Breeding systems in penduline tits: Sexual selection, sexual conflict and parental cooperation

PhD thesis

Ákos Pogány

Department of Ethology, Biological Institute, Faculty of Science, Eötvös University, Hungary

Supervisors

Prof. Tamás Székely (Department of Biology and Biochemistry, University of Bath, UK)

Dr. Vilmos Altbäcker (Department of Ethology, Eötvös University, Budapest, Hungary)

PhD School in Biology

Head of PhD School: Prof. Anna Erdei

PhD Program in Ethology

Head of PhD Program: Dr. Ádám Miklósi

– Budapest, 2009 –

Table of contents

I INTRODUCTION

1. Sexual conflict and sexually antagonistic coevolution

a) Sexual conflict: development of theory

For a long time, behavioural biologists and ethologists considered sexual reproduction as a joint venture of males and females, in which they cooperate to achieve their common aim beneficial to both of them: producing viable offspring that inherit and in turn pass over their genes to future generations (Arnqvist and Rowe, 2005). However, it is partly the process of genetic inheritance during sexual reproduction that contributed to the more recent realization that reproduction is not all that harmonious. In diploid organisms, the parents share approximately half of the offspring's genes, whereas in a large outbred population the male and female parents only share a small proportion of alleles due to common descent. This means that either parent may increase its reproductive success even if this is harmful to its mate. Indeed, males and females may face a conflict of interests in many aspects of reproduction (sexual conflict, Parker, 1979).

In an influential paper that would receive over 3800 citations, Robert Trivers (1972) was the first who drew attention to the different reproductive roles of the sexes. Following Bateman (1948), Trivers suggested that differences in investments to reproduction (i.e. sex differences in gamete production) leads to conflict of interests: females usually invest more heavily in gametes than males, and this drives females being more selective. The initial bias in investment would also influence efforts that each sex should make in later reproductive phases, especially in providing parental care for their offspring. Dawkins and Carlisle (1976) challenged Trivers' proposition – indeed, Trivers himself lamented that his original idea may not be watertight (Trivers, 1985) – and it initiated a series of theoretical researches focusing on sex differences in reproductive roles. Instead of past investment, other factors were identified which likely influence the outcome of sexual conflict e.g. the ratio of sexually receptive females and active males (Emlen and Oring, 1977); relative costs and benefits of parental care to each sex (Maynard Smith, 1977); or certainty of parentage (Queller, 1997).

Geoff Parker (1979) in his fundamental work clarified the concept of sexual conflict and introduced models of sexual conflict shaping the evolution of sexes. Using dungflies (*Scatophagidae*) as a model system, he was the first who provided evidence of

sexual conflict working in nature. Emphasizing sexually antagonistic coevolution resulting from different evolutionary interests between males and females, Parker (1979) placed sexual conflict within the framework of sexual selection. The general importance of sexual conflict in male-female coevolution was later highlighted by Holland and Rice (1998), Lessells (1999) and by the monograph of Arnqvist and Rowe (2005) among others, all widening the perspective and pointing out the general applicability of the theory.

b) Sexually antagonistic selection and the forms of sexual conflict

Individuals of a population in general do not entirely share their genes: they may have different alleles and these alleles compete with each other. Following this, the same applies for a breeding couple (Dawkins, 1976). Since male and female roles during reproduction differ substantially stemming from anisogamy (Parker et al., 1972), selection on morphological or behavioural traits often act in different directions on males and females. This may result in either an evolutionary tug-of-war over the same trait expressed in both sexes, or in an ongoing arms race of different traits expressed in males and females which influence the outcome of sexual conflict over a male-female interaction. These processes are termed sexually antagonistic selection, a potential consequence of conflict of evolutionary interests between the sexes (Lessells, 2006; Parker, 2006), whereby those attributes favoured in one sex are penalized in the other.

Intralocus versus *interlocus sexual conflict*

Whether sexually antagonistic selection results in an evolutionary tug-of war or in an arms race, may depend on the location of competing alleles on which the antagonistic selection acts. First, if a trait is determined by a gene at the same locus in the sexes, and selection favours different values for this trait as expressed in males and in females, intralocus sexual conflict emerges (Parker and Partridge, 1998). Selection on the trait in one sex therefore impedes adaptation in the other, and the observed average phenotypic trait value in the population is a compromise: none of the sexes may reach their optimum.

Evolutionary significance of intralocus conflict in male-female coevolution is controversial given that sex-limited expression should evolve rapidly to resolve this type of conflict. However, recent studies in *Drosophila* model systems identified intralocus sexual conflict as an important evolutionary drive in this species (Prasad et al., 2007; Price and Hosken, 2007). In *Drosophila spp*, genome modifications allow many genes transmitted solely through males and therefore selection in these modified strains can optimize male

function separately from that of female function. Biasing phenotypes towards male optima may have a fitness cost for females, thus intralocus conflict seems to prevent males from reaching their optima (Prasad et al., 2007; Price and Hosken, 2007).

Second, optimal outcome of any interactions may differ between males and females over reproduction (e.g. mating rate, female remating behaviour, infanticide, fertilization efficiency or parental effort; reviewed by Arnqvist and Rowe, 2005), and alleles influencing the interaction may be located at different loci in males and in females (interlocus sexual conflict, Parker and Partridge, 1998). The characteristics of interlocus sexual conflict are very different from those of intralocus sexual conflict. Since the involved alleles are not exposed to stabilizing selection by the expression in the other sex, rather the male phenotype and female phenotype evolves as a response to each other when trying to overrule the effect of selection in the other sex, the result is an ongoing antagonistic coevolution between the sexes (Gavrilets et al., 2001; Holland and Rice, 1998; Rice and Holland, 1997). From the two types of sexual conflict (intra- *vs.* interlocus) the latter appears to be a more powerful process shaping male-female coevolution. The ongoing arms race between males to manipulate, and females to resist male manipulations have shown to drive the evolution of morphological (Arnqvist and Rowe, 2002a) and behavioural traits (Chapman et al., 2003), and therefore it is an important process behind the evolution of sexual dimorphism and even speciation (Arnqvist et al., 2000; Parker and Partridge, 1998).

Pre-zygotic versus *post-zygotic sexual conflict*

The conflict of interest may emerge before or after fertilization takes place (pre-zygotic and post-zygotic sexual conflict, respectively, Royle et al., 2002). Pre-zygotic sexual conflict emerges over the number of mates and/or matings. Originating from the differential early investments to gamete production, the reproductive success of one sex (usually males) depends more on the number of mates, than that of the other sex (usually females, Bateman, 1948; Trivers, 1972). Although Snyder and Gowaty (2007) pointed out the methodological flaws of Bateman's original paper, recent studies using molecular techniques to establish genetic paternity support sexual selection fuelled by variance in male mating success (e.g. in the socially monogamous bird, the splendid fairy-wren, Webster et al., 2007; or in the green swordtail fish, Tatarenkov et al., 2008).

Pre-zygotic sexual conflict has profound influence on sexual interactions preceding matings. Males may theoretically increase their reproductive success by mating with many

females, whereas females may do so only by selecting good quality fathers to their offspring and avoiding costly and – from their perspective – unnecessary matings (Andersson, 1994; Arnqvist and Nilsson, 2000; Bateman, 1948). Therefore, males are selected to manipulate females by sexual signals or force them to achieve fertilization, whereas females are selected to resist the manipulation by being selective on prospective mates (Gavrilets et al., 2001; Holland and Rice, 1998). This pre-zygotic sexual conflict contributes to the evolution of various behavioural and morphological adaptations and counter-adaptations which ensure fertilization of a given male (e.g. mate guarding in different bird species, copulatory plugs in small mammals, morphological grasping traits, intrusive hypodermic insemination and genital damage in insects, sexual cannibalism in spiders), as well as avoidance of multiple matings and fertilization by low-quality males for females (e.g. anti-grasping traits, cryptic female choice, search for extra-pair copulations, reviewed by Andersson, 1994 and Arnqvist and Rowe, 2005).

Post-zygotic sexual conflict – besides infanticide (e.g. in lions) – often emerges over parental effort: in many species, care is beneficial for the young since it improves offspring survival, however, it is costly to the caring parent (in the forms of energy expenditure, increased mortality during care, and reduced future breeding success and/or survival, Balshine-Earn et al., 2002; Clutton-Brock, 1991). Therefore, each parent would benefit if the other takes a larger share in raising their young (Houston et al., 2005; Lessells, 1999). Depending on whether the resolution of conflict manifests *via* continuous or discrete decisions, the strategies that each parent may employ includes reduced parental effort (Houston and Davies, 1985), or providing no care at all (offspring desertion, Székely et al., 1996). There is a strong interaction between the evolution of parental care systems and breeding systems, in that offspring desertion allows the deserting sex to increase its future chance to reproduce (Houston et al., 2005; Roff, 2002; Wedell et al., 2006), and this includes reproduction in the same breeding season (social polygamy). In contrast, biparental care and monogamy restrict remating opportunities to search for extra-pair copulations (social monogamy, Reichard and Boesch, 2007; Székely et al., unpublished). The interaction between sexual conflict, sexual selection and breeding system evolution is further discussed in *Chapter 3* of Introduction.

The outcome of post-zygotic sexual conflict may depend on the behaviour of the mate, as well as the behaviour of other members of the population (game-theoretic approach, Houston and Davies, 1985; Maynard Smith, 1977). This is probably most apparent in species with highly variable breeding system, where either of the parents may

desert its offspring (e.g. snail kite *Rostrhamus sociabilis*, Kentish plover *Charadrius alexandrinus*, Eurasian penduline tit *Remiz pendulinus*, St. Peter's fish *Saratherodon galilaeus*, McNamara et al., 2002; Székely et al., 1996). Therefore, full understanding of care and desertion patterns requires a game-theoretical analysis that includes costs and benefits of care and desertion, and the process of interaction between parents (van Dijk et al., 2007; Webb et al., 1999; Webb et al., 2002). As both the costs of care and the benefits of increased chance for future reproduction are generally high, strong sexually antagonistic selection is expected to act on traits influencing the outcome of conflict over care (Chapman et al., 2003). For instance, in Eurasian penduline tits females burry their eggs during egg-laying in the bottom of the nest, and this behaviour was suggested to evolve by post-zygotic sexual conflict (Valera et al., 1997). The parent who first deserts puts its mate into a difficult situation: care is costly, but if the second parent to decide does not care, the clutch will certainly die. Therefore, it is in both of the parents' interests to keep their intentions of desertion in secret and try to desert first (van Dijk et al., 2007). For a male, desertion is beneficial only if he can be somewhat certain that his present clutch will result in offspring, and a possible sign of this is if his mate starts laying eggs. However, since females gain also if they desert (Szentirmai et al., 2007), keeping in secret that egg-laying has started may be a good tactic that increases the chance of a female to desert first (Valera et al., 1997).

2. Sexual selection

Darwin (1871) introduced the concept of sexual selection and he identified two processes which are responsible for the evolution of traits apparently not favoured by natural selection. Both intra- and intersexual competition happens between individuals of one sex (usually males) to achieve more matings by the other sex (usually females, Andersson, 1994; Darwin, 1871). These processes explain variance in mating and fertilization success, and the only difference between them is whether females participate in the process or not. In intrasexual competition (male-male competition), those males are favoured, which bear traits helping them to intimidate or defeat their rival males, whereas in intersexual competition (female choice), males compete with each other to be selected by females. The processes of intra- and intersexual competition may act in the same time on the evolution of a trait (either taking effect in the same or in contrasting directions, Wong and Candolin,

2005), so that the trait may be simultaneously involved in both male-male competition, and in female choice (dual function, Berglund et al., 1996).

a) Evolution of female preference

In contrast to the evolution of male-male competition, explaining and finding support for the evolution of female preference proved to be difficult. While the costs and benefits a male faces during intrasexual selection to gain access to females are straightforward and easy to show empirically, models of female choice are more complicated and competing theories are compatible. This makes empirical separation of the involved processes a challenging task (Andersson and Simmons, 2006; Kokko, 2001). Females may gain two kinds of benefits by choosing males possessing certain traits. First, she may get resources which improve her chance to survive and to produce offspring (e.g. good territory, food, parental care by the male). Since these benefits have direct effects on the female's survival and own reproduction, they are referred to as direct benefits, or non-genetic benefits. Second, the benefits by female choice may manifest only *via* her offspring's increased survival and reproduction, so that the female does not gain any resource herself. These are the indirect benefits or genetic benefits, which may be divided further into two main groups on the basis whether the male trait preferred by females reflects the quality of the male or not (honest signals evolving by indicator mechanisms, or Fisherian traits evolving by self-reinforcing runaway selection, Andersson, 1994). Additionally, female pre-existing bias that has evolved under natural selection for a different reason (e.g. in foraging context), may be responsible for the evolution of female preference (Fuller et al., 2005; Ryan, 1998; West-Eberhard, 1984).

Whichever types of benefits are the selection forces behind female choice (non-genetic benefits or genetic benefits), the common benefit of female mate choice is increased survival and/or attractiveness of her young (Jennions and Petrie, 2000; Kokko et al., 2002).

b) Male-female coevolution by sexual selection and sexual conflict

Intersexual selection and sexual conflict are often hard to be distinguished, since both of these evolutionary processes result in male-female coevolution: male ornamentation and female preference in sexual selection and manipulation and resistance in sexual conflict theory. However, there are important differences in the underlying selection forces that the male trait impinges on female fitness. In sexual selection theory, the male trait is preferred

because females gain direct or indirect benefits by selecting males with such traits, so that the male trait is advantageous to females. However, in sexual conflict theory (focusing on the conflict over mating), the trait that increases male mating success is disadvantageous to females, and resistance evolves as a counter-effect to decrease costs related to mating in females (Arnqvist and Rowe, 2005; Chapman et al., 2003; Gavrilets et al., 2001).

It is the same outcome of these two processes that makes separating sexual selection and sexual conflict often difficult. Furthermore, recent studies suggest that sexually antagonistic selection (i.e. sexual conflict) and different forms of sexual selection are not acting separately in nature, rather they work together – possibly with different relative strength in various stages of male-female coevolution. For instance, female 'preference' (i.e. resistance) may evolve by sexually antagonistic coevolution, but consequently females may gain indirect benefits by more grand-offspring from a good-manipulating male (Cordero and Eberhard, 2003). Nevertheless, indirect benefits may be less powerful than needed to counter-balance the often large direct costs of male manipulation in females (Cameron et al., 2003; Chapman, 2006; Chapman et al., 2003). A possible way to separate the mechanisms leading to male-female coevolution is to look at the selection forces resulting in female preference or resistance (Chapman et al., 2003; Kirkpatrick and Ryan, 1991; Kokko, 2001; Rowe and Day, 2006).

3. Parental cooperation *versus* sexual conflict

Competition and the 'selfishness' behind it are primary conditions for evolution (Dawkins, 1976). Since resources are often limited, and competitors are numerous, we expect to see a 'struggle for existence' over resources (e.g. food, territory, breeding site, Darwin, 1859). However, genomes, cells, multicellular organisms, animal and especially human societies all show different levels of cooperation (West et al., 2007). Cooperation is a behaviour by which an individual sacrifices some of his reproductive potential in order to increase that of another individual. This is in contrast with the process of natural selection, therefore special conditions and mechanisms have to apply which drive the evolution of these interactions towards cooperation (Nowak, 2006; Taylor and Nowak, 2007). Recently the origin and evolution of cooperative behaviour gained a renewed interest from evolutionary biologists (Colman, 2006; Nowak, 2006; Pennisi, 2005). Although the theoretical background has been well developed (West et al., 2007), empirical evidences supporting or

rejecting theories are scarce, and most of them focus on testing predictions of cooperative games e.g. different versions of the prisoner's dilemma or ultimatum game (Noë, 2006) in different animal and human groups (mainly in apes, Brosnan and de Waal, 2003; Jensen et al., 2007; lions, Heinsohn and Packer, 1995; Legge, 1996; wasps, Innocent and West, 2006; birds, Stephens et al., 2002 and humans, Kümmerli et al., 2007; West et al., 2006).

In the context of breeding systems, parental cooperation and sexual conflict can be considered as two contrasting strategies (Komdeur and Heg, 2005). While cooperation between parents leads to biparental care (social monogamy) and relaxed sexually antagonistic selection, sexual conflict may result in uniparental care (social polygamy) and intensified sexual selection (Figure I.1). In this view, both pure cooperation and pure sexual conflict are the extremes of the cooperation-conflict scale, and many populations are probably at an intermediate level. But what determines the position of a population along this axis? For answering this question we have to investigate the possible reasons and consequences related to the evolution of conflict and cooperation in natural reproductive systems (Figure I.1). Penduline tits (*Paridae, Remizinae*) are ideal subjects to investigate environment-sexual conflict-breeding system interactions, because they occupy very diverse environments varying from rich marshlands to poor semi-desert habitats, and they exhibit a variety of breeding systems ranging from intense sexual conflict with offspring desertion to cooperative breeding.

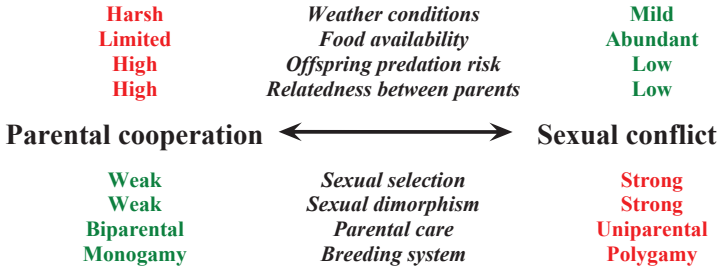

Figure I.1. The parental cooperation – sexual conflict axis of breeding system evolution. Some of the possible selection forces leading to increased levels of cooperation/conflict are listed above the arrow, and the expected outcomes of bias towards either direction are listed below the arrow. Red indicates more intense, more variable or more severe conditions, whereas green stands for less intense, or less variable conditions.

II STUDY SPECIES, OBJECTIVES AND METHODS

1. Ecology and breeding system of penduline tits

Penduline tits are small passerine birds (body length: 80-110 mm, weight: 6-12 g), with pointed, conical beak and characteristic, elaborate, basket-shape domed nest woven from plant downs, fibres and animal hair (Cramp et al., 1993; Harrap and Quinn, 1996). 13 species are included in the subfamily, most of them are included in two genera: *Remiz* and *Anthoscopus* (Monroe and Sibley, 1993), although classification of the group is still disputed (Alström et al., 2006; Gill et al., 2005). Apart from one species living in Central America (Verdin *Auriparus flaviceps*), penduline tits occupy East and Central Asia, Europe and Africa. They inhabit variable environments, from the poor and arid semi-desert fringes of Sahara (Sennar penduline tit *Anthoscopus punctifrons*) and Gobi deserts (White-crowned penduline tit *Remiz coronatus*) to the rich wetlands of Europe (Eurasian penduline tit *Remiz pendulinus*, Harrap and Quinn, 1996).

These ecologically variable habitats combined with variable breeding systems across the group make penduline tits ideal subjects to study sexual conflict, sexual selection, cooperation and their interactions with environment. My PhD thesis focuses on two penduline tit species, the Eurasian penduline tit *Remiz pendulinus* and the Cape penduline tit *Anthoscopus minutus*. The studies in my thesis concern the dynamic breeding system of Eurasian penduline tits (*Chapters III – VII*), and in *Chapter VIII* the breeding systems of Eurasian and Cape penduline tits are compared. These two species appear to represent the two endpoints of the sexual conflict – cooperation axis (Figure I.1), and I chose them to facilitate detection of the underlying processes and consequences of the contrasting breeding strategies.

a) Eurasian penduline tit: a model species of sexual conflict

The Eurasian penduline tit (Figure II.1) is one of the few species in which within-population variation in the breeding system was found (McNamara et al., 2002; Székely et al., 1996). The dynamic breeding system of this small passerine bird is sequential polygamy; females and males have up to 6 and 7 mates, respectively, in a single breeding season (Persson and Öhrström, 1989; Szentirmai et al., 2007).

Figure II.1. Nest-building Eurasian penduline tits. The pair (on the left) is building a nest in intermediate stage and the male (on the right) is bringing nest material to his almost finished nest.

Breeding systems across 5 European populations were similar (Franz, 1991; Persson and Öhrström, 1989; Pogány et al., 2008b) in which uniparental care was provided by females largely, at 47-65 % of nests, whereas males provided care at 6-18 % of nests, and the remaining nests (29-40 %) were deserted by both parents. Biparental care has never been observed in any of these studies, although it was rarely observed during short periods of feeding in other populations (Schönfeld, 1989; Schroth and Helbig, 1985).

A puzzling feature of the Eurasian penduline tit breeding system is the high frequency of apparently maladaptive biparental desertion. When both parents desert the offspring, the male wastes his time and effort on building the nest and attracting a female, whereas the female wastes her energies on egg-laying. A recent study by our group (Szentirmai et al., 2007) showed that biparental desertion is the result of an intense sexual conflict over parental care, since both sexes increase their reproductive success by desertion, however, this at the same time decreases the reproductive success of their mate. The uniquely diverse breeding system and studies investigating the evolutionary mechanisms behind it resulted in that the Eurasian penduline tit has recently become one of the few model species of sexual conflict theory (Arnqvist and Rowe, 2005).

Eurasian penduline tits are common breeders across Europe and Central Asia. Penduline tits occur all year round in Hungary, although the breeding population may be replaced by others during the winter (Cramp et al., 1993; Haraszthy, 2000). They have a long breeding season lasting from early April to August, and initially males arrive first to the breeding grounds which are along dykes and gallery forests of fishponds, lake shores

and river banks. Penduline tits build their nests mainly on willow (*Salix spp.*) and poplar (*Populus spp.*) trees using tightly woven threads of plant fibres (willow *Salix spp*, hop *Humulus lupulus*, nettle *Urtica dioica*, grasses), and nests are compacted tightly with plant downs (poplar *Populus spp*, reeds *Phragmites communis,* Cramp et al., 1993; see Figure II.1). Nests are initiated by the unpaired male and during the course of nest-building, he sings an elaborate song to attract females. Males usually suspend nest-building at an intermediate stage (stage C or D, Cramp et al., 1993; Figure II.1), and only if they acquire a mate, the male and female finish nest construction together. Some males, however, complete their nest even if they did not acquire a mate. Unmated males may abandon their nests and immediately start building a new one.

The egg-laying phase is initiated when the parents start building the entrance tube. From the second or third day of egg-laying onward, at least one parent deserts its offspring and current mate (Persson and Öhrström, 1989; van Dijk et al., 2007). If the male deserts the nest and the female stays, she continues egg-laying until up to seven eggs, whereas if the female deserts the clutch, the male is constrained to have a limited number of eggs, usually 3-4 eggs (*Chapter VI*). Incubation lasts for approximately 14 days, and the male or female feeds the young for about 21 days before they fledge (Cramp et al., 1993).

b) Cape penduline tit: parental cooperation

The Cape penduline tit (Figure II.2) shows a contrasting breeding strategy to that of the Eurasian penduline tit. Although the two species are close relatives, male and female Cape penduline tits are socially monogamous and have obligatory biparental care (Dean, 2005). They mate for one breeding season and they can have two breeding attempts together. The second brood is most often raised in the first nest of the pair, and fledged chicks from the first brood frequently stay with their parents as helpers-at-the-nest to assist raising the new young (facultative cooperative breeding, Dean, 2005).

The Cape penduline tit is a near-endemic, common resident in Southern Africa (South Africa, Botswana and Namibia). In Cape Province, the main breeding season starts in August and terminates around November (Á. Pogány, R. E. van Dijk and T. Székely, personal observation). Cape penduline tits pair up before the start of the breeding season. In contrast with Eurasian penduline tits, the male and female build the nest together from the beginning on the end of thin branches of trees and shrubs (Harrap and Quinn, 1996). The nest is similar in shape to the Eurasian penduline tit nest, although smaller in size. A major difference between the two species' nests is the false entrance at Cape penduline tit

nests, and that the spout of the nest can be closed (Harrap and Quinn, 1996; Figure II.2). By these two features, nest predation decreases some 5-6 times compared to other species breeding on the same ground (P. Lloyd, unpublished data). Females lay 3-6 eggs, which hatch after about 16 days of biparental incubation. Young are fed by both parents for approximately 24 days (Dean, 2005).

Figure II.2. Male Cape penduline tit and his nest in the fynbos vegetation of South Africa. The visible 'hole' of the nest is a false entrance with dead end; the birds open and close the flat entrance tube above it when entering or leaving the nest.

2. Objectives

The aim of my thesis is to test predictions of sexual selection and sexual conflict theories of male-female coevolution. I focus on three main topics:

− the evolution of sexual signals *via* sexual selection and sexual conflict;

− different sex roles and their influence on the outcome of post-zygotic sexual conflict; and

− breeding system evolution by sexual conflict and cooperation.

a) Sexual selection and pre-zygotic sexual conflict in Eurasian penduline tits

According to sexual selection theory, females may gain direct and/or indirect benefits by mating with attractive males. An alternative explanation is provided by sexual conflict theory, suggesting that females 'prefer' (i.e. resist to) male manipulative traits because male manipulation is disadvantageous for them. In the first part of my thesis, my aim is

two-fold. First, I test which male traits of Eurasian penduline tits are involved in sexual selection. Second, I use field data to test which of the two contrasting mechanisms (classical sexual selection or sexual conflict) is more likely to have shaped the evolution of male mask size by investigating possible female costs related to this male trait.

Male traits involved in sexual selection in Eurasian penduline tits

Previous field studies indicated that nest size and male mask size may be involved in female mate choice, and that mask size may also signal in male-male competition (Grubbauer and Hoi, 1996; Hoi et al., 1994; Kingma et al., 2008; Schleicher et al., 1996; Szentirmai et al., 2005b). In *Chapter III*, I investigate female preference for mask size and nest size using an aviary choice test, and in *Chapter IV* and *V*, I test whether mask size plays role in male-male signalling in an aviary and in a field experiment, separately. *Chapter V* also investigates acoustic signalling in male-male competition and in engaging to extra-pair copulations by females.

Separating sexual selection and sexual conflict in the evolution of male mask size

The competing theories of sexual selection and sexual conflict theory have similar outcomes: they both result in male-female coevolution of male secondary sexual signals and female choosiness. However, the underlying selection mechanisms acting on females are very different in these two processes. While in sexual selection females are selected to choose certain males for the benefits they gain, in sexual conflict females 'choose' between males to avoid being manipulated by them (i.e. male trait is associated with a cost for females). In *Chapter VIII*, I test whether male mask size is costly for females in terms of parental care, thus preference for mask size and the consequent parental decisions are consistent with predictions of sexual conflict or sexual selection theory.

b) Sex differences and post-zygotic sexual conflict in Eurasian penduline tits

Previous studies have shown intense sexual conflict over parental care in Eurasian penduline tits. Although either sex may care for the young, studies from five European populations showed a bias toward female care. My objective was to investigate possible reasons leading to the observed care patterns.

Are females better parents than males?

Female-biased uniparental care in Eurasian penduline tits may be interpreted as males 'winning' the conflict over care more often than females. However, alternative explanations, such as the parental quality hypothesis (Eckert and Weatherhead, 1987; Erckmann, 1983) need to be addressed too. If males and females differ in the quality of their provided care, the better caring sex (females) could be under natural selection to care more frequently than the other sex (males). I investigate this assumption in *Chapter VI*.

Flexibility of parental care decisions in Eurasian penduline tits

There are three ways for within-population variance over parental care to emerge, and investigating which of the three processes is involved may shed light on the evolutionary mechanism responsible for breeding system evolution. First, individuals may either care or desert their subsequent nests consistently, thus within-population variance builds up by between-individual variance. Second, each individual may vary randomly in their decisions at subsequent nests regardless of environmental influence. Third, individuals may vary, but in line with changes in their environment. In the last two cases, the variable breeding system builds up by within-individual variance. In *Chapter VII*, I investigate which of these processes is responsible for the variable breeding system of Eurasian penduline tits.

c) Implications of sexual conflict and cooperation on male-female coevolution

Sexual conflict theory predicts that sexually antagonistic selection on males to manipulate and females to resist such manipulations should result in male-female coevolution: sexual dimorphism, and sexually different behaviours and roles during reproduction should evolve. By comparing the breeding systems between closely related species, one with intense sexual conflict (Eurasian penduline tit) and one with extensive cooperation (Cape penduline tit), I investigate the impact of sexual conflict and cooperation on the morphology and behaviour of these two penduline tit species in *Chapter VIII*.

3. Methods

The studies included in this thesis were carried out on different organization levels (individual – population – species level), and I used three different approaches throughout the thesis: aviary experiments (*Chapters III* and *IV*), field experiments (*Chapter V*) and

field observations (*Chapters VI – VIII*). In all experiments (*Chapters III – V*), I have participated in all stages of and done most of the work, whereas data collection for studies involving field observations (*Chapters VI - VIII*) has been done in collaboration of other students and fellow researchers.

a) Aviary experiments

In order to investigate the effects of different male traits on female preference and male dominance rank, in the first year of my study I established a captive population of Eurasian penduline tits in Göd Biological Station of Eötvös University (22 km from Budapest, 47º40'N, 19º07'E). Using mist net and song playback, 40 penduline tits were caught in total on 26 September 2003 and 19 October 2004, during the autumn migration in Hortobágy fishpond, Hungary (47º38'N, 21º05'E). Permissions to capture penduline tits were provided by Hortobágy National Park (refs: 84-93/2003 and 22-433/2004), and permission to keep penduline tits in captivity was provided by Duna-Ipoly National Park (ref: 6658/2/2003). Penduline tits were housed in purpose-built indoor and outdoor aviaries (for details on keeping conditions, see *Chapter 1* of Appendix).

All penduline tits were ringed with one numbered metal and two coloured rings (A C Hughes, Middlesex, UK) which allowed unambiguous identification of the birds. Standard morphometric measurements, including tarsus length, wing length and body mass were taken (see details in Bleeker et al., 2005; Kingma et al., 2008). Moreover, three digital photographs were taken of each side of the bird's head using an Olympus FE-100 and a Fujifilm FinePix A203 digital camera. In all photographs we kept a ruler in the background to be able to measure mask size. The birds were hand held touching the ground and the camera was positioned at an approximately fixed distance (about 20 cm) from the bird to standardize aberrations. The area of the mask (to the nearest 0.01 cm^2) was quantified from the digital photographs using Adobe Photoshop 7.0 software (Adobe System Inc., San Jose, California, USA). Mask size was calculated as the average values measured on the three pictures.

Sexing penduline tits

Eurasian penduline tits have sexually dimorphic plumage thus they can be sexed based on their morphological traits: males have large black masks and brighter overall colouration allowing unambiguous determination of their sex (Cramp et al., 1993; Kingma et al., 2008). Nevertheless, our captive population was also sexed from blood samples using the

CHD gene (Griffiths et al., 1998) at the University of Bath; all 40 penduline tits provided unambiguous results. Dissection of 10 penduline tits that died of natural causes provided fully consistent results with molecular sexing.

For molecular sexing 5-10 µl blood was taken from the left wing vein following standard blood sampling protocol. The vein was first punctured with sterile insulin syringe, and heparinized microcapillaries were used to collect the precise amount of blood sample (Assistant Disposable Micropipettes 555/50, Karl Hecht GmbH & Co. KG, Germany). Blood sampling did not have any adverse effect on the birds.

b) Field observations and experiments

Fieldwork was carried out in two sites; Eurasian penduline tits were investigated in Fehértó, Hungary whereas Cape penduline tits were investigated in Koeberg, South Africa.

Fehértó fishpond system, Hungary

Fehértó is an extensive fishpond system in Southern Hungary (46°19'N, 20°5'E; area: 1321 ha), consisting of 16 fishpond units which are separated by dykes (Figure II.3). This wetland habitat accommodates one of the biggest breeding populations of Eurasian penduline tits in Europe, with 158-183 nests yearly in which 60-90 males and 45-50 females breed (min-max, based on data collected between 2002-2007). Permissions to do fieldwork in Fehértó was provided by Kiskunság National Park, Hungary and Alsó-Tisza-vidéki Környezetvédelmi, Természetvédelmi és Vízügyi Felügyelőség, Hungary (refs: 577-3/2002; 390-2/2003; 1094/2004; 23864-1-1/2005; 23864-2-3/2006; 23864-3-2/2007).

Nest-building males were searched for and nests were checked approximately every other day throughout the breeding season, and males were mist netted when building their nest using song playback and a male penduline tit dummy (Bleeker et al., 2005; Szentirmai et al., 2005a). Female penduline tits were caught either together with their mate during mist netting, or they were caught in the nest during incubation using a specially designed nest-trap (Z. Barbácsy, personal communication). Penduline tits were ringed with a numbered metal ring of the Hungarian Ornithological Institute, and three colour rings (A. C. Hughes, Middlesex, UK) that allowed us to identify the individuals from a distance using binoculars. Morphometric measurements and digital photos were taken following the same methodology as described above. Nests of mated pairs were checked approximately daily. Desertion by the male and/or the female was established if the given individual was not observed at the nest for 15 min on two consecutive days (van Dijk et al., 2007).

Figure II.3. Typical habitat of the Eurasian penduline tit. The dykes which separate the fishponds in Fehértó are edged by reed, poplar and willow trees. These provide ample food (insects) and nest sites for penduline tits.

Koeberg Nature Reserve, South Africa

Cape penduline tits were investigated in the coastal scrubland (fynbos vegetation, Figure II.4) at the 572 ha Koeberg Nature Reserve near Cape Town, South Africa (33º40'S 18º26'E). 10 and 11 nests were followed, at which eight and six breeding pairs were monitored in 2006 and 2007, respectively. The low number of monitored nests in Cape penduline tits is due to the low population density in this species, as large territories are used by family groups (Dean, 2005).

Cape penduline tits were trapped in their nests, as the couple spend the night together inside the nest. A thin hair-net was applied around the entrance tube before dawn, so that the couple got caught when emerging for the first time after sunrise. The same standard morphometric measurements were taken as for Eurasian penduline tits, and individuals were ringed by a metal ring from the South African Bird Ringing Unit and a unique combination of three colour rings (A.C. Hughes, Middlesex, UK). Digital photographs were also taken from the birds' plumage, following the methodology described above.

Figure II.4. Cape penduline tits live in fynbos habitat in Koeberg Nature Reserve, South Africa.

III FEMALE CHOICE IN THE PENDULINE TIT *REMIZ PENDULINUS*: THE EFFECTS OF NEST SIZE AND MALE MASK SIZE

Abstract

Why do females prefer some males over others? Females often use multiple cues, and to distinguish between these cues one needs to manipulate putative male traits. We carried out a test of multiple cues hypothesis in a polygamous bird, the Eurasian penduline tit. In this passerine both males and females mate with up to seven mates within a breeding season, and a single parent (male or female) incubates the eggs and raises the chicks. Males build sophisticated nests, and previous studies suggested that females prefer males with large nest to small ones, since large nests provide direct benefit to females *via* reduced cost of incubation. Males sport wider eye-stripes ('masks') than females, and males with large masks find a mate faster than males with small masks. In a mate choice experiment using factorial design and two levels for each trait, we show that females prefer males with large masks, whereas they do not show preference for large nests. These results suggest that in penduline tits (i) females pay more attention to a trait that signals indirect benefit (mask size) than a trait that is related to direct benefits (nest size), and (ii) nest preference may be context-dependent.

Ákos Pogány, Tamás Székely, 2007. *Behaviour* 144:411-417.

Introduction

Females may draw two kinds of benefits from choosing a particular male: they may gain direct benefits (or 'non-genetic benefits') such as food, good territory or paternal care, or indirect benefits (or 'genetic benefits') such as 'good genes'. Depending on whether the preferred trait signals the quality of the father or not ('honest signal' or 'Fisherian trait'), the benefits may manifest by increased survival or attractiveness of her young (Andersson, 1994; Jennions and Petrie, 2000; Kokko et al., 2002).

We investigated experimentally the mate choice of the Eurasian penduline tit. Both direct and indirect traits appear to be involved in female choice in this species. First, males build elaborate pendulous nests to attract females. Nest-building takes considerable time and effort: in a Hungarian population monitored between 2002-2004, nests were built for about two weeks (mean ± SE: 16.4 ± 0.7 days), and during the building period males spent about 16 % of their time on collecting nest material and building their nests. Nests are made of tightly woven threads of plant fibres and they have excellent insulating abilities; traditionally, they were used as padding materials in boots during cold winters in Hungary. Nest size correlates with the thickness of the nest, and thick nests reduce the cost of incubation (Grubbauer and Hoi, 1996; Hoi et al., 1994; Szentirmai et al., 2005b). Therefore, females gain direct benefits by choosing males with large nest, and female penduline tits are predicted to use nest size as a potential cue in mate choice (Hoi et al., 1994; Schleicher et al., 1996).

Second, male penduline tits have larger melanised eye-stripes ('masks' henceforth) than females (Cramp et al., 1993; Kingma et al., 2008), and males with a large mask mate sooner than males with small mask (Kingma et al., 2008). Melanin-based ornaments had been considered to signal mainly social status, whereas recent works suggest that melanised ornaments are also involved in female choice (Bókony et al., 2003; Dale, 2006; Griffith et al., 2006; Jawor and Breitwitsch, 2003; Tarof et al., 2005). Two further findings support mask size as female cue in penduline tits: mask size is more variable between males than non-ornamental morphological traits (such as tarsus length), and mask size is not involved in male-male competition (Kingma et al., 2008). Moreover, male mask size does not correlate with territory quality in penduline tits (Brinkhuizen & wa Kang'eri, unpublished report, University of Groningen), thus Kingma et al. (2008) suggested that females gain good genes or attractive sons by mating with large mask males.

Multiple cues often provide different information on male quality (Candolin, 2003; Møller and Pomiankowski, 1993; Siefferman and Hill, 2003; Zuk et al., 1992). Distinguishing between cues is not straightforward (but see Calkins and Burley, 2003; Dale and Slagsvold, 1996; Lendvai et al., 2004), since some traits may signal direct, whereas others indirect benefits. We conducted an aviary experiment to investigate the role of both traits (nest size and mask size) in female choice of penduline tits. We predicted that females should prefer males with a large mask and/or large nest. Our experimental design allowed us to asses the relative significance of these traits, and their interactions.

Methods

Birds and aviary conditions

We investigated the choice of 16 female penduline tits that were kept in five same-sex indoor aviaries. To avoid stress during breeding period, from 30 April until 30 September the penduline tits were also held in six outdoor aviaries. We used a 7-17 hour regime (dark - light) for six weeks prior to and during the experiment to facilitate the females' reproductive activity (Lambrechts and Perret, 2000).

Penduline tits kept their good health, condition and body weight throughout the experiment. Since we planned further experiments with our captive population, they were not released at the end of experiment.

Choice apparatus

The experiment was carried out between 17 May 2005 and 1 July 2005 in Göd Biological Station using a purpose-built indoor choice apparatus (Figure III.1). The size of choice apparatus was 3.4 m x 2 m x 2.4 m, and consisted of three chambers: two choice chambers ('A' and 'B'), and a neutral 'Middle' chamber. We provided two perches each in A and B, and one perch in Middle. The female was separated from the dummy and the nest by wire mesh. We conducted pilot trials with two females when the mesh was removed. Since neither female entered the inner area of A and B that was previously fenced off, we used the mesh in all trials (see Figure III.1). The female was only able to see the dummy and the nest and approach them up to 20 cm, if she entered A or B. The chambers were lit by commercially available spot lights (3 x 60 W in each chamber). During pre-test sessions only the Middle chamber was lit, whereas during test session only chambers A & B were lit to encourage the female to visit the choice chambers.

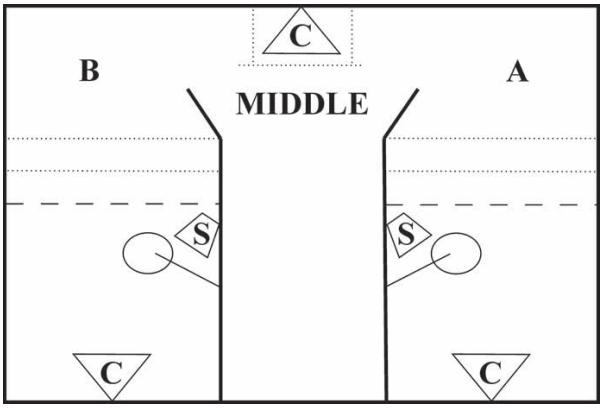

Figure III.1. Schematic view of the choice apparatus we used for the female choice experiment in penduline tits. The choice chambers 'A' and 'B' are separated by a no-choice area ('Middle'). Open circles indicate nests and dummies, dotted lines indicate perches, and dashed lines indicate wire-mesh. Male song was played in both A and B from speakers (S) and the location of cameras are given by 'C'.

Male penduline tits sing to attract females whilst building their nest, and to simulate natural mate choice situation as closely as possible, we played male song in both choice chambers (Figure III.1). The same song was played in both A and B using two systems each consisted of a compact disc player (XP-V730, Aiwa Co. Ltd, Japan), an amplifier (PA-100, Monacor GmbH & Co. KG, Germany) and a 3-way speaker (MKS-58/SW, Monacor GmbH & Co. KG, Germany). Male songs were recorded in Southern Hungary, and we used two 30 min recordings. The order of these records were swapped between A and B, so that in total the same songs were played in both chambers during a one-hour trial. The behaviour of females was tape-recorded using three security video cameras (Videosec W-101, Euro Tech Corporation Kft, Hungary; Figure III.1). A 4-channel quad processor (AVC713, AV Tech Corp., Taiwan) projected the pictures of three cameras on one screen, and the images were saved on a video recorder (LG Electronics Inc., Korea).

Experimental design

We investigated the effects of mask size and nest size in a factorial design using hand-painted plaster male dummies and naturally deserted penduline tit nests. Each trait had two levels (small and large; Figure III.2). We had two sets of small-large pairs of nests and dummies, and we used a randomly selected set for a given trial. The dummies were

prepared using an identical mould, and the only intentional difference between these dummies was their mask size. Similar penduline tit dummies have been used in the field to mist net penduline tits, and males react to these dummies as to real intruding males (Kingma et al., 2008). Abandoned penduline tit nests were collected in Fehértó, Southern Hungary (46º19'N, 20º05'E; see details in Szentirmai et al., 2005b) after the breeding season in late August and September, 2004. Since females visit prospective males during nest-building, to mimic situations when mate choice occurs naturally we only collected nests that were in appropriate stage (stage C: see Cramp et al., 1993, pages 385-387; Figure III.2), or if the collected nest was in a more advanced stage we removed nest material to resemble to stage C as closely as possible. Nest height and mask size were different between small and large treatment groups (*t*-tests: nest height: $t_2 = 5.27$, $P = 0.034$; mask size: $t_2 = 6.064$, $P = 0.026$; Table III.1), and both traits were within the natural ranges (Table III.1).

Figure III.2. An example of male dummies and nests used in the choice experiment: small mask and a small nest (on left), and large mask and large nest (on right). Note that we used four dummies and four nests altogether (see Methods).

Table III.1. The sizes of nests and masks used in the mate choice experiment of penduline tits. Two sets of dummies and nests were randomly used to control for possible dummy-effect. Ranges are given for natural mask size and nest size for males and nests, respectively, in Fehértó, Hungary. Our measurements of nest size and mask size followed the methodology of Szentirmai et al. (2005b) and Kingma et al. (2008), respectively.

	Nest height (mm)	**Nest width (mm)**	**Nest bottom thickness (mm)**	**Mask size (cm^2)**
Small set 1	140	82	24	1.02
Small set 2	150	74	14	0.93
Large set 1	185	86	49	1.54
Large set 2	200	106	30	1.41
Natural range	*120-190*	*70-150*	*4-64*	*0.996-1.90*

During trials one choice chamber always contained a small nest and a dummy with small mask ('Small/Small' henceforth, where the first term indicates nest size and the second term corresponds to the mask size), while the other choice chamber contained one out of four factorial treatments ('treatment side' henceforth). We controlled for possible confounding effects by randomizing female order, treatment order, treatment side and song type order (see below). Side effect (A *vs* B) was also controlled by swapping nests and dummies between chambers (see below).

On Day 0 the female was acclimatized to the chambers for two hours when one live male was presented in each choice chamber (Figure III.1). On Days 1-4 females were subjected to one out of four treatments between 9 a.m. and 3 p.m. (Central European Time) using the following protocol. **Session 1**: Rest period: the female was released into the Middle chamber and then was left to relax for 30 minutes. **Session 2:** Trial period with presenting the stimuli for 60 min. **Session 3:** Rest period for 30 min while the dummies and nests were swapped between chambers A and B. **Session 4:** Trial period for 60 min with treatments being swapped between A and B.

Two females were tested on each day, so that female 1 was tested during Sessions 1-4, and female 2 was tested during Sessions 5-8. The order of females was randomized. Sessions 1, 3, 5 and 7 were termed pre-stimulus periods, whereas Sessions 2, 4, 6 and 8 were termed stimulus periods.

Decoding video tapes

We measured the times the female spent in each chamber using a chess-clock (Aradora, Romania), and the counter of the video recorder. The chess-clock was calibrated to the counter of the videotape to provide consistent times. One of the chess clocks was dedicated to measuring time spent in A, and the other to measuring time spent in B. When the female was in Middle, both clocks were off, and the time spent in Middle was calculated by subtracting time in A + B from the total session time. Change in percentage times was calculated between each stimulus (%) and its corresponding pre-stimulus period (%) for chambers A and B:

$$[1] \quad \Delta A = A_{stimulus} - A_{pre\text{-}stimulus}$$

$$[2] \quad \Delta B = B_{stimulus} - B_{pre\text{-}stimulus}$$

Preference was calculated as the difference between ΔA and ΔB:

$$[3] \quad Pref_1 = \Delta A - \Delta B \qquad \text{if treatment is in A and Small/Small is in B,}$$

$$[4] \quad Pref_2 = \Delta B - \Delta A \qquad \text{if treatment is in B and Small/Small is in A.}$$

Finally, the preference was calculated as the average of $Pref_1$ and $Pref_2$ to cancel out side effects. Note that Small/Small was also included among treatments, so that it was also compared against the reference Small/Small.

Data processing

To compare the behaviour of females between stimulus and non-stimulus periods, we wrote a permutation test in R 2.2.1 (R Development Core Team 2005, Vienna, Austria). In permutation tests first we calculated the *t* test statistic (t_{crit}) as given by Sokal and Rohlf (1995). Then permutated the dataset 10^4 times, and for each permutation we calculated the *t*-statistic. We provide the proportion of cases when the *t*-value was larger than t_{crit}. Since this was a two-tailed test, the absolute values of *t* and t_{crit} were used.

The effects of nest size, mask size and their interaction on female choice were investigated using Generalized Linear Mixed Models (GLMM) in SPSS 11 (SPSS Inc., Chicago, USA). Two models were used: GLMM1 included mask size, nest size and daytime as fixed factors, and female ID as random factor. GLMM2 included the same components, however, one female was excluded from this model (see below). Since nest size x mask size was not significant, we did not include this term in either of the models.

Results

Female behaviour during stimulus versus *pre-stimulus periods*

Females spent significantly more time in chambers A and B during stimulus periods than during pre-stimulus periods (random permutation tests: chamber A, t_{crit} = 15.87, P < 0.0001; chamber B, t_{crit} = 13.91, P < 0.0001, Figure III.3). Consistently, females spent less time in the Middle chamber during stimulus periods than pre-stimulus periods (t_{crit} = 24.92, P < 0.0001).

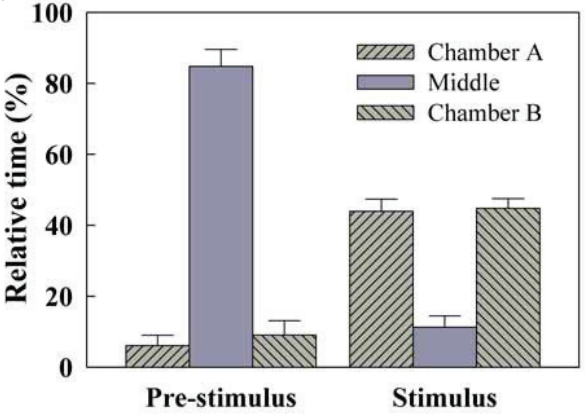

Figure III.3. Percentage of time female penduline tits spent in each chamber during pre-stimulus and stimulus periods (mean + 1 SEM). Females spent significantly more time in both A and B during stimulus periods than during pre-stimulus periods (see Results).

The effects of nest size and male mask size

We found a weak female preference for dummies with large mask, but found no preference for nest size (GLMM1: *mask*: $F_{1,45}$ = 3.072, P = 0.086, *nest*: $F_{1,45}$ = 0.433, P = 0.514, *female ID*: $F_{15,45}$ = 2.101, P = 0.028; *daytime*: $F_{1,45}$ = 3.997, P = 0.052; Figure III.4; Table III.2). The interaction between nest size and mask size was not significant ($F_{1,45}$ = 0.311, P = 0.580), therefore we did not include it in our final model.

Three out of eight outliers were due to a single female (see Figure III.4). After excluding this female with extreme responses from the GLMM, the effect of mask size became highly significant, and neither nest size nor daytime related to female response (GLMM2: *mask*: $F_{1,43}$ = 7.550, P = 0.009; *nest*: $F_{1,43}$ = 1.504, P = 0.227; *female ID*: $F_{14,43}$ = 1.763, P = 0.078; *daytime*: $F_{1,43}$ = 2.586, P = 0.115; Table III.2).

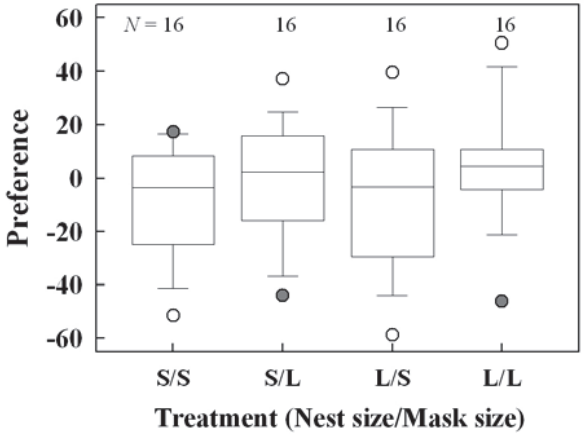

Figure III.4. Female preference (see Methods). The box-plots indicate the median, 10th, 25th, 75th and the 90th percentiles. The circles indicate outliers outside the 10th and 90th percentiles. Three out of eight outliers (indicated by dark circles) were due to a single female. S: small, L: large nest and/or mask size.

Table III.2. The effects of mask size and nest size on female preference in penduline tits (Generalized Linear Mixed Model with mask size, nest size and daytime as fixed factors, and female identity as random factor, see Methods). Two models are given. In the first model all females ($N = 16$) were included, whereas from the second model one female that provided three outliers, was excluded (see Figure III.4).

	GLMM1 ($N = 16$ females)			**GLMM2 ($N = 15$ females)**		
	F	***DF***	***P***	***F***	***DF***	***P***
Mask size	3.072	1	0.086	7.550	1	0.009
Nest size	0.433	1	0.514	1.504	1	0.227
Female ID	2.101	15	0.028	1.763	14	0.078
Daytime	3.997	1	0.052	2.586	1	0.115

Discussion

Our study provided two major results. First, we showed experimentally that female preference was influenced by mask size of male dummies in the penduline tit. Although this effect was only a trend in the main model using the traditional 0.05 critical level, by removing one female with extreme behaviour, the preference became statistically highly significant. Second, female penduline tits did not show preference for large nests. Thus mask size, a trait that appears to be associated with indirect benefits, is a more important cue than nest size, a trait that is linked to direct benefit.

Our experimental results from the aviary corroborates the findings of a field study in which mask size influenced mating time of males (Kingma et al., 2008). The mechanism by which intersexual selection acts on this trait is not apparent. Since melanised pigments are considered cheap to produce, we would expect sexual selection to act in a Fisherian way. Earlier experimental manipulations are consistent with this assumption, and suggest that melanised ornaments are not condition-dependent (Hill and Brawner, 1998; McGraw et al., 2002). However, recent findings showed that melanised ornaments may signal body condition thus act as honest signals (Griffith et al., 2006; Parker et al., 2003; Siefferman and Hill, 2003). Thus, the benefit that females may gain by choosing males with large mask need further investigations to reveal the underlying mechanisms.

In nature, females may cue on male building behaviour and she may contribute more to a good-builder's nest than to a nest of poor builder (differential allocation, Sheldon, 2000). These confounding effects may hamper the interpretation of nest size and female choice relationships in nature. However, in our experiment both small and large nests were in the same stage during choice trials and females did not contribute to the nests. Therefore, we were able to investigate the pure effect of nest size without the confounding effects of female or male building effort (Hoi et al., 1996; Persson and Öhrström, 1996).

We found no evidence that nest size is involved in female choice. This result is consistent with a field study of Hungarian penduline tits (Szentirmai et al., unpublished data) that did not find female preference for large nests. These works, however, are in contrast with previous field studies where females preferred large nests over small ones (Hoi et al., 1994; Schleicher et al., 1996). We suggest two potential explanations for these conflicting results. First, preference for large nests may be weak, therefore our sample size might have been too small to detect preference. Second, female preference for well-insulating nests may be context-dependent, thus manifest only when ambient temperature

is low. Recent theoretical and empirical studies of female preference suggest that changing environmental conditions attribute significantly to the variance in female preference for a particular male trait (Cotton et al., 2006; Qvarnström, 2001). In our case, the constant temperatures inside the aviaries and in the choice apparatus might have kept preference for nest size suppressed. Females incubate the eggs for approximately 16 days, and internal and external nest temperatures are substantially different especially early in the breeding season. This predicts that female preference for well-insulating nests should be more pronounced (i) early in the season than later on (Schleicher et al., 1996), (ii) during cold breeding seasons than mild ones, and (iii) at higher latitudes than lower ones. The first two of these predictions are testable using our choice apparatus by manipulating ambient temperature in order to test whether female preference is plastic in regards to environmental conditions.

Besides environmental influence, variation in female choice may involve heritable variation in trait preference (Brooks and Endler, 2001; Jennions and Petrie, 1997; Widemo and Saether, 1999). In our experiment, we found different responses between females to stimuli. Thus it seems plausible that some of these between-female differences are due to heritable effects.

The influence of multiple cues in female choice has been investigated in various organisms recently (e.g. Hamilton and Sullivan, 2005; Kodric-Brown and Nicoletto, 2001; Lendvai et al., 2004). To our knowledge, our study stands out by testing the effects of traits that representing both direct and indirect benefits, separately. To understand the underlying mechanisms of female choice, one needs to clarify the types of benefits signalled when multiple cues are involved. Thus we need to follow up this line of research by comparing the benefits from direct *versus* indirect traits in the penduline tits (Arnqvist and Kirkpatrick, 2005; Hadfield et al., 2006).

Song is a sexually selected trait in many passerines (Ballentine et al., 2004; Catchpole, 1996; Tomaszycki and Adkins-Regan, 2005). Indeed, male penduline tits sing vigorously whilst building their nest. Whereas in the current experiment we used song simply to raise the interest of the female, we plan to manipulate song traits to assess their significance in female choice. It will be valuable to assess not only the song features in isolation from other traits, but to assess its relative importance, for instance in regards to mask size.

Our experiment using male dummies appears to have two limitations. First, female penduline tits may not perceived the dummies' masks as a natural trait, since birds have

different visual perception from humans (Bennett et al., 1994; Bennett and Thery, 2007; Endler, 1990). However, we investigated the effect of an achromatic, melanised patch that has strong light absorbance in the whole spectrum, and black plumage typically has little or no reflectance in the UV spectrum (Bennett et al., 1994; McGraw, 2006). Therefore, we argue that the dummies' mask substituted realistically the masks of live males. Second, by using dummies we excluded the influence of male behaviour on female choice. This limitation may be severe, because in nature males court and sing vigorously, so it is possible that their behaviour (rather than their physical trait such as mask size or the features of their nest) may influence mate selection by females. These propositions can be tested by manipulating male behaviour in aviary, for instance, by hormone implants (Adkins-Regan, 1999; Foerster and Kempenaers, 2005).

In conclusion, we showed that female penduline tits use mask size as a potential mate choice cue, and they do not exhibit preference for large nests in our aviary experiment. These results suggest that a trait that signals indirect benefits elicited stronger response than a trait related to direct benefits. Given the unusually diverse breeding system of penduline tits, and the possibility that nest preference may depend upon the ambient environment, further experiments will be illuminating to clarify cues the females use in assessing potential mates, and the precise benefits they derive from such a choice.

IV MALE SIGNALLING AND DOMINANCE IN THE PENDULINE TIT *REMIZ PENDULINUS*

Abstract

Secondary sexual characters (e.g. badges, ornaments) are involved in many species in male-male competition and/or in female choice. We tested a proposition, the armament-ornament model in a small passerine bird, the Eurasian penduline tit, which exhibits an unusually diverse breeding system that includes sequential polygyny and polyandry, and uniparental care by the male or the female. Previous studies showed that the size of black eye-stripes (masks) in males functions as an ornament, since females prefer males with large masks. Here we report that in an aviary experiment, male mask size did not predict dominance status. Our results are consistent with a field study, and these together suggest that male mask size is a secondary sexual signal in penduline tits: it is a trait used by females in mate choice decisions.

Ákos Pogány, Vilmos Altbäcker, Tamás Székely, 2008. *Acta Zoologica Academiae Scientiarum Hungaricae* 54:191-199.

Introduction

The evolution of secondary sexual signals is one of the most puzzling aspects of evolutionary biology, ever since Darwin (1871) described the theory of sexual selection. Darwin separated two versions of sexual selection to explain how traits not favoured by natural selection may evolve. By intrasexual selection (mostly between males), those males are favoured which bear traits ('armaments' henceforth) helping them to defeat or intimidate their rivals, and by their means achieve more access to females than other competing males (e.g. black badges of Eurasian siskins *Carduelis spinus* and great tits *Parus major*; reviewed by Griffith et al., 2006; Senar, 2006). Whereas *via* intersexual selection (mostly females choosing males), those males are selected that exhibit certain traits that are preferred by females ('ornaments' henceforth); these traits may indicate direct or indirect benefits for females (e.g. beak colouration of zebra finches *Taeniopygia guttata*, male pheromones of the sandfly *Lutzomyia longipalpis*, reviewed by Andersson, 1994; Jennions and Petrie, 2000 and Andersson and Simmons, 2006).

Berglund et al. (1996) argued that many secondary sexual signals have dual functions, i.e. serve both as armament (such as weapons and status badges) and ornament. The duality might give rise to new theories about signal evolution, and the interaction between competition and mate choice (armament-ornament model, Berglund et al., 1996; Wiley and Poston, 1996). However, the latter interaction, the issue whether competition and mate choice are parallel (or contrasting) processes is controversial (reviewed by Wong and Candolin, 2005).

Black masks of male Eurasian penduline tits may have dual function. On the one hand, experimental and field studies showed that male mask size is involved in female choice (Kingma et al., 2008; Pogány and Székely, 2007; but see Schleicher et al., 1996). On the other hand, several features of penduline tits suggest that male mask size may be involved in male-male competition. First, the penduline tit mating system is one of the most diverse of avian breeding systems, with sequential polygamy (up to seven mates in a breeding season by both males and females), and uniparental care by either sex (Franz, 1991; Franz and Theiss, 1983; Persson and Öhrström, 1989; Szentirmai, 2005). Second, a recent study focusing on sexual conflict over parental care revealed that either sex may maximize its own reproductive success by deserting their mate and clutch, and breeding with new mates (Szentirmai et al., 2007). Therefore, both female choice and male-male competition are expected to be amplified. Third, unmated males react aggressively towards intruder males, and they attack dummy males presented close to the residents' nests. This

behavioural response is exploited for trapping and ringing males during fieldwork (Bleeker et al., 2005; Persson and Öhrström, 1989; Szentirmai et al., 2007).

Here we report an aviary experiment which was designed to investigate the role of male mask size in male-male competition. Our prediction was that male penduline tits establish dominance rank based on their relative mask size, hence male mask size will predict their dominance status.

Methods

Birds and aviary conditions

We investigated the dominance behaviour of nine male penduline tits, using two female penduline tits as stimuli. Male and female penduline tits were housed together in two same-sex indoor aviaries outside the breeding season (between 15 September and 31 March). During the breeding season (1 April to 14 September) males and females were housed in pairs in five indoor and six outdoor aviaries. Males were monitored before 1 March for possible signs of their breeding stage (chasing, singing and nest-building, Á. Pogány, personal observation), and they exhibited breeding behaviour (courting for females, singing and nest-building) from late April onwards. After 1 April the experimental males did not meet apart from the trials (see below), so that they could not possibly establish dominance rank prior to the experiment.

Breeding activity

A 7–17 hour regime (dark – light, Lambrechts and Perret, 2000), and extra Lisovit-R combi vitamin supply (Pentarex Bt, Érd, Hungary) was used for six weeks prior to and during the experiment to facilitate the reproductive activity of males and females.

Between 25 May 2005 and 4 June 2005 the behaviour of five experimental males were monitored to determine whether they were in breeding condition. Each male was observed five times on five consecutive days for 30 min randomly either in the morning (8 a.m. – 11 a.m.) or in the afternoon (2 p.m. – 5 p.m.). The observer recorded the frequency of singing and nest-building. Then we calculated the number of singing in an hour and the proportion of time the males spent with nest-building. Males were in breeding stage prior to the experiment, since the five males we observed in detail exhibited both nest-building and singing behaviour (they spent 1.20 ± 0.44 % of their time with nest-building, and sang in average 7.60 ± 7.27 times in an hour).

Competitive context of the experiment

Males exhibited sexual behaviour during trials (see below). We noted that four out of nine males sang, and sometimes males chased each other. Moreover, each of the nine experimental males approached the female's stimulus cage during trials (mean % of time spent on the perch and the mesh: 8.77 ± 1.32 %), while without female in the stimulus chamber and during acclimatization, males have never been observed to stay in the proximity of stimulus chamber (i.e. on perch or mesh, Á. Pogány and A. Meredith, personal communication).

Morphometric measurements

Morphometric measurements (tarsus length, wing length, body mass) of males were taken. Body condition was calculated as residuals from linear regression of body mass on tarsus length. Photos for mask size calculation were also taken. We use absolute mask size, because mask size is unrelated to body size in penduline tits (Kingma et al., 2008). Mask asymmetry was also calculated, as the absolute value of (mean left size – mean right size)/mean right size.

Dominance apparatus

The experiment was carried out between 14 June 2005 and 19 June 2005, in a purpose-built dominance apparatus (Figure IV.1). The size of the apparatus was 3.4 m x 3.4 m x 2.4 m, and it consisted of two regions that were separated by wire-mesh. In the smaller partition ('stimulus chamber' henceforth), a female was placed to stimulate the males to compete with each other. The larger partition ('arena' henceforth) was marked into six equal partitions (L1-L3, R1-R3) by reed pots. Wood sticks were fixed to the floor at the edges of each partition for assisting the localization of males. We distinguished five distance categories: mesh (0 cm) – when male settled down on the part of wire-mesh that separates stimulus chamber and arena, perch (12 cm) – this perch was fixed on the wire mesh, L1-R1 (middle point: 45 cm) – first partition of arena both on the left and right sides, concordantly, L2-R2 (middle point: 135 cm), L3-R3 (middle point: 225 cm) partitions of the arena.

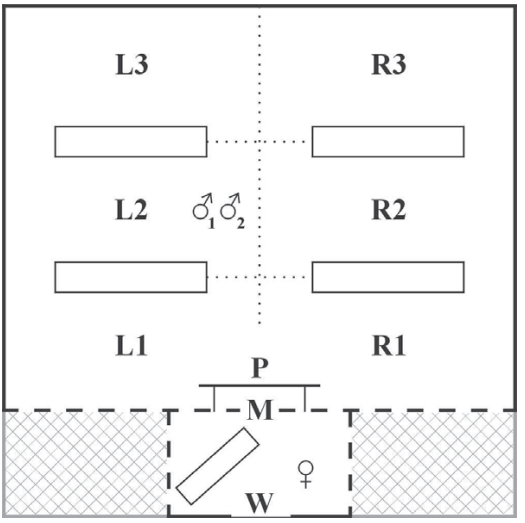

Figure IV.1. Schematic view of the apparatus used in dominance tests for male penduline tits. A female penduline tit was placed in the stimulus cage which was separated from the males by wire-mesh (dashed lines). In each trial two males were released in the arena. The arena was divided into six equal squares (three on the left side L1-L3 and three on the right side R1-R3) by pot reeds (rectangles). Trials were observed through a one-way window (W). Dotted lines indicate wood sticks on the floor that assisted the allocation of distance categories. Food and water was provided during experimental trials in pots placed in the middle point of the arena, i.e. between L2 and R2. Size of room was 3.4 m x 3.4 m x 2.4 m.

Trials were monitored through a one-way detective window by adjusting light conditions properly, therefore the experiment could be followed without disturbing the birds. This setup allowed us to distinguish and identify males unambiguously using their colour rings.

Experimental design

We investigated male dominance in a fractional factorial design (Box et al., 2005). Nine males were randomly allocated into three groups. In each group all males were tested once with his other two groupmates separately, using a female as stimulus. Trial order within blocks was randomized. After all males in a group were tested (three trials), the female was replaced by a new one, and trials were repeated to control for the possible female-driven effect. Thus each male-pair was tested in two trials, and we averaged their responses.

In each day one group was tested with one stimulus female, so that we had three trials per day. Twenty minutes before each trial, the two males were released in the arena to let them acclimatize. Then the female was released into the stimulus chamber, and the two males' distance from the female was estimated in every 20 second for one hour. The positions of the two males were noted in the larger partition at each time interval. Neither the males nor the female were familiar with the experimental apparatus before their first trial.

Statistical analyses

For each pair of males we calculated their difference in mask size, mask asymmetry, tarsus and wing length, and body condition. We used two response variables: proximity was calculated as the relative average distances of the males from the stimulus female, whereas endurance was estimated by the relative times of males spent in the vicinity of female. In endurance 'vicinity' was defined as the total time spent on the perch and mesh, see Figure IV.1. We assumed that a lower value in proximity and/or a higher value in endurance indicate higher dominance status.

The relationships between morphometric traits and proxy variables of dominance were tested using SPSS 11 (SPSS Inc., Chicago, USA). We provide mean ± SE, the Pearson correlation coefficients (r), and two-tailed significance levels.

Results

Variation in male mask size

In the three experimental groups of males, mask size was 1.29 ± 0.07 cm^2, 1.22 ± 0.07 cm^2 and 0.82 ± 0.07 cm^2 (N = 3 in each group, mean ± SE). Variance in mask size was comparable across groups: 0.015, 0.017, and 0.016 cm^2, respectively.

Morphometrics and dominance

Proximity was unrelated to the differences between males in any trait including mask size (r = -0.315, P = 0.409, N = 9), mask asymmetry (r = 0.243, P = 0.528, N = 9), wing length (r = -0.468, P = 0.204, N = 9), and body condition (r = -0.507, P = 0.164, N = 9).

Endurance was also unrelated to differences between males in mask size (r = 0.301, P = 0.432, N = 9), mask asymmetry (r = -0.084, P = 0.830, N = 9), wing length (r = 0.122, P = 0.755, N = 9), and body condition (r = 0.136, P = 0.728, N = 9).

Discussion

We found no evidence that male penduline tits use mask size as an armament cue in a competitive context. Therefore, in line with our earlier study on the effect of mask size on female preference, we suggest that male mask size is a secondary sexual trait more likely involved in female choice than in male-male interactions.

This experiment and a female choice experiment (Pogány and Székely, 2007) contribute to a small number of studies that tested the duality function, and found support only for the ornament function (Berglund et al., 1996). Other studies however, found results consistent with dual functions (Berglund et al., 1996; Griggio et al., 2007; Jones and Hunter, 1999). For instance, Tarof et al. (2005) showed black masks are involved in both male-male competition and female mate choice in common yellowthroat *Geothlypis trichas*, a small passerine with similar black mask to that of the penduline tits. We propose three explanations for the different results between Tarof et al. (2005) and our ones as presented here. First, mask size as a secondary sexual signal may have evolved *via* intersexual selection in penduline tits, and may have no function in male-male competition. This proposition is corroborated by a field study of Kingma et al. (2008) in which mask size of males was unrelated to aggressive response to intruder-mimicked situation.

Second, since Eurasian penduline tits appears to be expanded from the Far East (Harrap and Quinn, 1996), where the presumed ancestors and close relatives exhibit little (or no) sexual dimorphism, it is possible that mask size as a cue in sexual selection is an evolutionarily recent phenomenon and had no time to evolve into a second function. The armament-ornament model (Berglund et al., 1996), although suggesting a more likely direction from armaments to ornaments, does not exclude that a sexual signal with dual function evolves by intersexual selection, and only later it gains a role in male-male competition.

Third, we believe our experiment provided a competitive situation for males, although it is difficult to prove this. Note, that our experimental males showed breeding behaviour before and during the experiment, and in each trial both males approached the stimulus female. Behavioural observations of non-experimental males confirmed that some of the males sang and chased each other during the experiment, and the experiment was carried out during their natural breeding season. Males react aggressively towards each other – even without apparent stimuli – both in nature and in aviary during their breeding season (Bleeker et al., 2005; Szentirmai et al., 2007; Á. Pogány, personal observation).

In the current study we only used 9 individuals due to constraints on sample size. If sample size can be increased to above 40 birds, $r = 0.3$ would reach significance level at $P = 0.05$, so it is a possibility that a larger experiment may provide a different conclusion.

We propose that male penduline tits may use other than morphological traits (such as mask size) when establishing dominance rank in competitive situations. For instance, song (Grubbauer, 1995), or behavioural traits are likely candidates for such cues.

In conclusion, our aviary experiment found no relation between mask size and dominance between males. These results, coupled with aviary experiments and field studies suggest that in penduline tits, mask size does not predict the outcome of male-male interactions.

V ACOUSTIC SIGNALLING IN EURASIAN PENDULINE TITS *REMIZ PENDULINUS*: THE EFFECTS OF INTRUDER SONG ON BEHAVIOUR OF RESIDENT MALES AND FEMALES

Abstract

Acoustic signals may have evolved to assist choosing a mate and/or to protect a territory. In many bird species songs are variable, and different characteristics of song, for instance repertoire size and song type, may have a separate function. We investigated the influences of song repertoires and song types on behaviour of resident Eurasian penduline tits in an intruder-mimicing situation. This small passerine bird (body mass is about 9 g) has variable breeding system that includes sequential polygamy by both sexes and uniparental care by the male or the female. Using song playbacks, we challenged resident males both before and after mating to test whether males respond differently (i) to large *versus* small repertoire sizes, and (ii) to different types of intruder song in Southern Hungary. We found that resident males did not respond differently to different repertoire sizes and song types, although their responses were predicted by their own repertoire size. Males reacted more intensely to intruders before than after pair formation, whereas females were unresponsive to playbacks. Our results therefore suggest that song in penduline tits is involved in male-male competition as intruder penduline tits may size up the resident's defensive response by his repertoire size.

Ákos Pogány, Otília Menyhárt, Timothy J. DeVoogd, Tamás Székely. *Manuscript*

Introduction

Acoustic signals are among the most common sexually selected traits in many taxa, especially in insects, anurans and birds (Andersson, 1994). With their complex songs, male passerine birds display towards conspecifics mostly in the breeding season (Catchpole and Slater, 2008; Searcy and Andersson, 1986). Males may communicate towards both sexes; first, if song is directed towards same-sex individuals, it may either signal the owner's quality and ability to defend territory boundaries against intruder males (Krebs et al., 1978; Searcy and Nowicki, 2000). Conversely, intruders may signal their quality to resident males. In the latter case, resident males are expected to react stronger to intruders with large repertoire than to those having small repertoire ('male quality hypothesis', Lambrechts, 1992). Second, male song may attract females, signalling male quality and/or stimulating females to mate (Catchpole et al., 1984; Catchpole and Slater, 2008; Searcy, 1992). In addition, male song may bear information in both intra- and intersexual communications, thus playing a role simultaneously in male contest and female mate choice (dual function, Berglund et al., 1996).

In many songbirds, song may be divided into two different song types (Catchpole and Slater, 2008; Kroodsma, 1982). Type I songs, which are shorter and simpler, are suggested to be involved in male-male communication during male contests. The simplicity and shortness of this type of song facilitates communication with other males. The function of type II songs, on the other hand, is to attract females thus this type of song is more elaborate and longer to increase the chance for a female to hear the continuous male song when passing by his territory. Experimental studies that focused on song types and their functions however, produced conflicting results (e.g. Ballentine et al., 2004; Beebee, 2004; Poesel and Dabelsteen, 2006; Trillo and Vehrencamp, 2005).

Male song may influence female behaviour in different ways: it may facilitate her breeding status, attract her from a distance or stimulate her for copulation (Kroodsma and Byers, 1991). Females may pick up specific characteristics of the males' song that may influence their behaviour in different stages of their reproductive cycle. For instance, Searcy (1992) hypothesized that mated females pay attention to other males' repertoire size than that of their social mate so that large repertoire size helps to achieve extra-pair copulations for males.

In this study, we investigated experimentally acoustic communication in Eurasian penduline tits. Males in this species sing elaborate songs, restrict singing to reproductive periods, and repertoire size varies between males: each of them sing multiple versions of

songs (O. Menyhárt, personal communication), suggesting that song is involved in sexual selection. Earlier field studies and laboratory experiments on sexually selected visual signals are in line with the dynamic breeding system of penduline tits, and reported visual signals to be sexually selected (mask size: Kingma et al., 2008; Pogány and Székely, 2007; nest size: Grubbauer and Hoi, 1996; Hoi et al., 1994; Schleicher et al., 1996; but see Pogány and Székely, 2007).

Extra-pair copulations by both male and female penduline tits are frequent and approximately 16 % of chicks are sired by other than the putative parents (Mészáros et al. unpublished). Although penduline tits do not have fixed territory size, nest-building males chase away intruder males near their nests. This behavioural response is probably driven by competition for nests and females in males, since 5.6 % of nests are taken over by intruder males at some stage of nest-building in our population (Á. Pogány, R. E. van Dijk and T. Székely, unpublished data). In some of these takeovers, the female stayed with the new mate, so that resident mated males may lose both their nest and mate to an incoming new mate.

Here we report a field experiment in which we investigated the role of male repertoire size (large *versus* small) and song type (type I *versus* type II) in male contest situation and female mate choice for extra-pair copulation. We predicted that resident males should react more intensively to playbacks with large repertoire, with type I song and after they acquired a mate. In the latter case, besides their nest they have their mate to lose too, and/or an increased chance for sperm competition. We also tested whether the resident males' reaction to intruders is predicted by their own qualities (as indicated by repertoire size or mask size). In addition, we tested whether females of resident males respond differently to playbacks, specifically, whether they approach more readily playbacks with large repertoire.

Methods

Field site and study population

The experiment was carried out in Fehértó between 22 May and 6 July in 2006. This period coincided with the peak breeding season of penduline tits (Szentirmai et al., 2005a). 26 males were included in the study ('resident males' henceforth): these males were mist netted when they built their first nest in the study site and they were ringed and digital photos of their masks were taken. Trapping took place 10.82 ± 2.67 days (mean ± SE)

before the first experimental playback (see below) of a given male, so that trapping unlikely influenced the outcome of the experiment.

Most females were unringed at the time of manipulation (two ringed females out of nine) since females are generally more difficult to catch with mist net than the males. We believe that these seven unringed females were likely different individuals given the size of our breeding population (40-50 females breed per year). Moreover, one unringed female whose test preceded the others' tests cared for her young while the other six were tested. From the remaining six unringed females, three were tested on the same day while mated to different males, decreasing further the possibility of pseudo-replication.

Song playbacks

Playbacks were compiled from male penduline tit songs recorded in 2005 in Fehértó, using a Marantz PMD 660 portable digital recorder and a parabolic microphone. Each male was recorded for approximately four hours, and songs were drawn from different individuals. From these recordings of different males, we compiled two sets of stimulus playbacks (set 1 and set 2) to control for limited number of stimuli (Kroodsma, 1989). Each set included four playback songs: a small and a large repertoire size playback ('intruder repertoire size' henceforth) and a type I and type II playback ('intruder song type'), respectively. Each song had the same total number of syllables, however, in both set 1 and set 2 playback songs, large repertoire size consisted of more different kinds of syllables. All of these songs included the most frequently used syllables, which were observed in most of the males' songs and thus they were considered as essential syllables (O. Menyhárt, personal observation).

To compile type I and type II playbacks, we selected monotone, repetitive syllables only (type I playback) and highly variable, trill syllables only (type II playback) for both sets of playback songs, respectively. In each repertoire size and song type playback 30 seconds of song was followed by a 30-second break to mimic natural singing behaviour of penduline tits. This series was repeated 10 times, so that total length of each trial was 10 minutes, using different syllables in each of these artificial singing bouts. Playback songs were compiled using Avisoft-SASLab Light v. 3.74.

Repertoire sizes of resident males

At 22 out of 26 males included in the study, we recorded approximately 4 h song from each in order to estimate natural repertoire size ('resident repertoire size' henceforth). This

recording time is sufficient to estimate repertoire size in Eurasian penduline tits (see *Chapter VIII*). Recording took place in each case before the first experimental trial of a given male. During these recordings, the parabolic microphone and digital recorder were hidden in the vegetation some 5 m from the male's nest, so that recording did not have any adverse effect on the birds.

Experimental design

Unpaired resident males were tested in two treatments for the effects of intruder repertoire size and song type. Both treatments had two levels; either small repertoire or large repertoire size, or type I – type II in song type, resulting in a total of four trials per male. At their first trial, either set 1 or set 2 playback was assigned to each male randomly (see above), and for all later trials of the same male, the same set was used consistently. On day 1 and day 2, males were subjected to two out of four trials each day, one trial between 6 a.m. and 10 a.m. (Central European Time, 'morning session' henceforth) and the second trial between 4 p.m. and 8 p.m. ('afternoon session').

At the first morning trial of a given male, treatment order (repertoire size or song type) was randomized, as well as treatment level order (small *versus* large repertoire or type I *versus* type II song type). At the second (i.e. the first afternoon) trial of a given male, treatment order was balanced, so that if his morning trial was repertoire size treatment he was subjected to song type treatment and *vice versa*, with treatment levels again randomized. On day 2 (morning and afternoon) each male was tested in a balanced design by following the treatment order – but with opposite treatment levels – of day 1 treatments. Thus each male was tested for repertoire size or song type trial pairs in either two consecutive mornings or afternoons, separately, starting in the same time. In each morning and afternoon session, males were tested in blocks of four: the order of the males within their first morning session was randomized, and then the same order was kept in their following three sessions. All experimental trials were initiated only when either visual or acoustic signs indicated that the subject in question is at the nest.

Mated males received a repeated repertoire size treatment (5^{th} and 6^{th} trials) on two consecutive days (both trials either in two consecutive mornings or afternoons, selected randomly), using the same set of playbacks that was used when the male was unmated to investigate the change in his response according to change in mating stage. We repeated only repertoire size treatments, as we intended to minimize disturbance at the nest. During the trials at mated pairs, we also monitored the behavioural response of females. In most

mated trials (five out of nine nests), the male was tested at a different nest when mated than that of his unmated nest; this was a consequence of rapid changes in nest location of unmated males, since males frequently abandon their nests to start building a new one without acquiring a mate at their earlier nest (Persson and Öhrström, 1989; Szentirmai et al., 2007; van Dijk et al., 2007).

Experimental protocol

Stimulus songs were played from 15 meters of the nests of resident males using a 3-way speaker (MKS-58/SW, Monacor GmbH & Co. KG, Germany) connected to an amplifier (PA-100, Monacor GmbH & Co. KG, Germany), a compact disc player (XP-V730, Aiwa Co. Ltd, Japan) and a lead acid battery power supply (LA 1220, Hi-Watt Co. Ltd, China). A hand-painted, plaster male penduline tit dummy, mounted on a stick, was placed immediately above the speaker (Figure V.1). The experimenter hid behind a hide (27 m from the nest), so that only the speaker, the dummy and the cord (hidden in the grass or reed) were positioned between the experimenter and the nest, whereas all other technical equipments were behind the experimenter and unlikely disturbed the resident birds. The direction of stimuli from the nest was randomized, except if characteristics of the surrounding vegetation were different on the two sides of the tree, when the experimenter chose the side according to visibility to be able to better follow the residents during trials. After the first trial of a male, the exact position of the stimulus speaker and dummy was marked, and all later trials were initiated from the same spot.

Figure V.1. Resident male penduline tits react aggressively towards playback songs and a dummy male penduline tit. Behavioural responses included attacking, i.e. pecking at the dummy, as can be seen on this photo.

The density of vegetation around the nest may influence the responses of resident tits to playbacks. To control for visibility, we estimated 'coverage' (0-3) for each site; 0 indicated that visibility was very good and the experimenter could see the nest from behind the hide, score 1 indicated that the nest could not be seen but visibility was sufficient between the stimulus and the tree of the nest, whereas scores of 2 and 3 indicated that visibility was sufficient only up to 10 m and 5 m from the stimuli, respectively.

Data processing and statistical analyses

We recorded the responses of resident males every 20 seconds of a 10-minute trial using two variables: (i) distance to stimuli (speaker and dummy), and (ii) behaviour during playback. First, we estimated the position of the male (and female) from the dummy and the speaker along x-y-z coordinates, with 0.5 m accuracy. To estimate distance accurately, before the first trial at a given nest we measured the distance of natural signs (e.g. a small bush or a trunk) from the stimuli or placed small wood sticks to indicate 5 m and 10 m from the dummy and speaker. These marks together with the height of the reed helped us to estimate the subjects' distance in 3-D, so that in each time interval distance from the stimuli was estimated as 3 coordinates along a virtual x-y-z coordinate system. Approach distance (d) from the stimuli was calculated for each time interval by calculating the length of the hypothetical diagonal of an x-y-z-sided cuboid:

$$[1] \qquad D = \sqrt{x^2 + y^2 + z^2}$$

Finally, we calculated the average *D* over the 10-minute trial.

Second, we noted the following behavioural responses: calling, singing, attacking the dummy and tail quavering (the latter is a signal of excitement by moving the tail feathers rapidly from side to side). These four behavioural units are probably related to sexual and aggressive situations in penduline tits (Á. Pogány, R. E. van Dijk, T. Székely, personal observation). Finally, we calculated the percentage of intervals when a resident male showed any of these behaviours, as a behavioural response to the intruder situation.

Our experimental design was not a full factorial design (see above) due to time constraints, thus we analysed the responses in four separate Generalized Linear Mixed Models (GLMM) in R 2.6.1 (R Development Core Team 2005, Vienna, Austria), each focusing on either the effects of intruder repertoire size or song type, and on different response variables. First, we restricted the analyses to unmated males and focused on repertoire size treatment using either approach distance to stimuli or frequency of

behavioural responses as response variables, separately. Since distribution of approach distance was not normal and skewed towards zero, we log-transformed distances ($\log_{10}$ (x + 1)) and used the transformed data in the model. % behavioural responses were also normalized by arcsine transformation for GLMM analysis. In models of repertoire size, playback repertoire size was included as fixed factor, whereas male ID was the random factor. Second, for song type models we used the same two response variables (approach distance and % behavioural response, separately), and built up the models similarly to repertoire size models, including playback song type as fixed factor and male ID as a random factor. In all four initial GLMMs we included the potentially confounding effects of time of day (morning *versus* afternoon), advance of the breeding season (measured as the number of days from 1, April), vegetation coverage around the nest and number of trial for the residents. Non-significant effects were eliminated stepwise, and we provide statistics for each excluded variable before its exclusion from the model.

Since male ID had to be included in our models as a random factor to control for repeated sampling, effects of the resident males' own quality (mask size and repertoire size) could not be investigated in the same models because of co-linearity. Male response in light of their own mask and repertoire size is therefore analyzed separately by linear regressions, in which average male response (approach distance or % behavioural response, separately) during the unmated trials (1-4) is regressed on either mask size or own repertoire size. We had mask size and repertoire size measurements for a subset of males involved in this study (mask size: $N = 21$ males; own repertoire size: $N = 22$ males) and this resulted in varying sample sizes between analyses.

Nine males out of 26 acquired mates, so we compared the responses whilst a male was unmated and mated using paired-samples *t* test. In these tests, the two unmated trials for repertoire size treatment were averaged for a given male, and compared to his average response on small and large repertoire trials when mated. Mated trials of two (out of nine) males preceded their unmated trials at their subsequent nest.

In all GLMM models and statistical tests two-tailed probabilities are given and we rejected the null-hypothesis at $P < 0.05$ level. We provide mean ± SE.

Results

Responses of resident males and females

All resident males (N = 26) responded to the intruder by approaching the dummy, and displaying at least one of four key behaviours (Table V.1). Unmated males were present around their nest in 59.48 ± 4.35 % of time during trials. Attacking (Figure V.1), the most aggressive behavioural reaction was displayed by four out of the 26 experimental males.

Females of resident males, however, did not seem to respond to playbacks in general. Whereas males were present in 44.28 ± 7.23 % of time during the mated trials, females were present in 3.00 ± 2.11 % of time (Table V.1). Three out of nine females approached the stimuli for short periods, although they did not show the focal behaviours of this study.

Table V.1. Responses of resident male and female penduline tits to song playbacks. Mean ± SE presence during trials, and responses (approach distance and % behavioural responses) are given. Unmated (UM) males were tested in repertoire size and song type treatments, whereas mated (M) males and females were only tested in repertoire size treatments.

	Repertoire size			**Song type**
	UM males	M males	M females	UM males
	N = 26	N = 9	N = 9	N = 26
% presence during trials	59.48 ± 4.35	44.28 ± 7.23	3.00 ± 2.11	61.24 ± 4.09
Approach distance (m)	5.51 ± 0.58	9.23 ± 1.83	10.54 ± 3.33	5.66 ± 0.67
% behavioural response	59.44 ± 3.61	31.11 ± 6.41	0	64.36 ± 3.36
% calling	20.30 ± 2.47	11.30 ± 3.09	0	24.94 ± 2.98
% singing	17.92 ± 2.86	6.67 ± 2.17	0	18.62 ± 2.47
% tail quavering	20.00 ± 3.07	12.96 ± 5.38	0	20.14 ± 2.80
% attacking	1.22 ± 0.65	0.19 ± 0.19	0	0.65 ± 0.30

Responses to repertoire size treatment

Unmated males did not approach differentially the stimuli with large and small repertoires (GLMM, $F_{1,25}$ = 0.129, P = 0.722), however, we found strong individual differences in approach distance (χ^2_1 = 15.621, P < 0.001).

Consistently, behavioural responses were not different to large *versus* small repertoire playbacks ($F_{1,25}$ = 0.178, P = 0.677), and each male responded to treatment levels highly consistently (χ^2_1 = 7.961, P = 0.005).

None of the investigated confounding variables (advance of the breeding season, time of day, number of trial and vegetation coverage: all $P > 0.191$) had significant effect on either approach stimuli or behavioural responses.

Responses to song type treatment

Playbacks with type I and type II songs did not elicit different approach from resident unmated males (GLMM, $F_{1,20} = 1.840$, $P = 0.190$), and approach distance was consistent between males ($\chi^2_1 = 10.060$, $P = 0.002$).

After controlling for time of the day (less behavioural response in the afternoon; time of day: $F_{1,20} = 4.423$, $P = 0.048$), male behaviour was similar in response to type I and type II songs ($F_{1,19} = 1.439$, $P = 0.245$), and consistent between trials ($\chi^2_1 = 3.941$, $P = 0.047$). Apart from time of day in behavioural response analysis, none of the investigated confounding variables had significant effect (see above, all $P > 0.105$).

Repertoire size and mask size of resident males

Mask size of resident males did not predict approach to stimuli (least squares linear regression: $b = 0.099$, $t = 0.433$, $P = 0.670$), or the percentage of behavioural responses ($b = 0.174$, $t = 0.771$, $P = 0.450$).

Although repertoire size did not predict approach distance ($b = -0.198$, $t = 0.902$, $P = 0.378$), it predicted the percentage of behavioural responses ($b = 0.567$, $t = 3.075$, $P = 0.006$; Figure V.2).

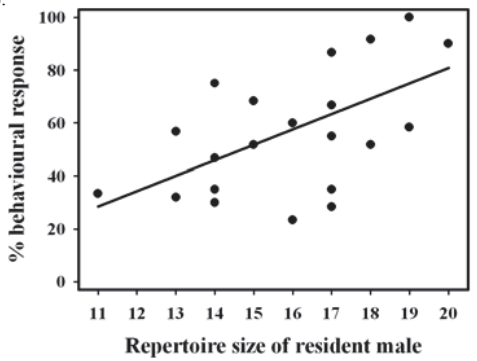

Figure V.2. Percentage of behavioural response (calling, singing, tail quavering and attacking) towards an intruder in relation to the resident male penduline tit's own repertoire size. Behavioural responses of 22 males given to small and large repertoire treatments and type I and type II songs were averaged and regressed on the resident male's repertoire size (least squares linear regression: $b = 0.567$, $t = 3.075$, $P = 0.006$).

Resident mating status and the strength of response

Males did not approach differently the stimuli before and after they acquired a mate (paired-samples *t* test, $t_8 = 1.717$, $P = 0.124$), however, they showed weaker behavioural response to playbacks after mating ($t_8 = 2.402$, $P = 0.043$; Figure V.3). This difference is unlikely explained by changes of male behaviour with advance of the breeding season, since mated trials were taken relatively close in time to unmated trials (8.33 ± 4.78 days, mean ± SE; *t* test of days from 1 April, unmated *versus* mated trials: $t_{16} = 1.337$, $P = 0.200$), compared to the approximately 3-month long breeding season of penduline tits (Harrap and Quinn, 1996).

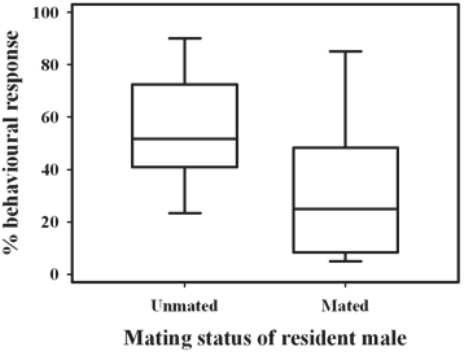

Figure V.3. Percentage of behavioural responses to intruders and mating stage in penduline tits. The two unmated trials for repertoire size treatments were averaged, and compared to the same male's average response on small and large repertoire treatments when mated. The box-plots indicate the median, 10th, 25th, 75th and the 90th percentiles. None of the data points were outliers (i.e. fell outside the 10th and 90th percentiles).

Discussion

Our results suggest that acoustic signals are involved in male-male competition in penduline tits. Males reacted to song playback close to their nest by approaching the stimuli and giving behavioural responses. Intruder repertoire size or intruder song type did not elicit different responses from resident males, however, we found strong and consistent individual differences in the strength of behavioural responses. These individual differences were predicted by the resident males' own repertoire size, suggesting that the direction of acoustic communication between males is from the resident towards the intruder.

Responses given to repertoire size treatments did not support the male quality hypothesis (Lambrechts, 1992), in line with other studies testing predictions of this hypothesis (Balsby and Dabelsteen, 2001; Catchpole, 1989; but see Stoddard et al., 1988). Likewise, intruder song type treatments did not elicit different responses from resident males. Functionality of different song types vary extensively between species, and our findings suggest that type I and type II songs in penduline tits do not have distinctive functions (Beebee, 2004; Weary et al., 1992). However, we acknowledge that our study measured only two possible response variables (approach to stimuli and behavioural responses), therefore we can not exclude the possibility that resident males might have reacted differently to treatments, for instance by song type matching (Beecher et al., 2000; Krebs et al., 1981) or song type variant switching (Searcy et al., 2000).

Resident males with large repertoire size gave more behavioural response towards the playbacks, so that resident males advertise their competitive will to defend their nest/mate by their repertoire size. Our challenging experiment revealed that this acoustic signal is an honest signal in male-male competition in penduline tits, and possible intruders may size resident males up according to their repertoire size (Beebee, 2004; Krebs et al., 1978; Nowicki et al., 1998; Yasukawa, 1981).

Mask size of resident males did not predict the strength of response to possible intruders. The results of our study – in light with earlier findings (Kingma et al., 2008; Pogány et al., 2008a) – suggest that acoustic signalling plays more important role than visual signalling in intrasexual communication in male penduline tits.

Contrary to our *a priori* prediction, resident males showed less interest towards an intruder after they acquired a mate than before. This suggests that the value of a given nest and nest site decreases after a resident male secured matings with a female. High rates of extra-pair paternity (Mészáros et al., unpublished) and female behaviour during our mated trials may provide an explanation to this pattern. Females spend very little time around the nest, and the couple move separately from one another when mated (van Dijk et al., 2007). Mate guarding has never been observed in this species (Á. Pogány, R. E. van Dijk, T. Székely, personal observation), therefore, it seems more likely that resident male penduline tits suffer from lost paternity through females encountering with other males at their nest than from the ones trying to take over their mate's nest. On the other hand, as time of desertion approaches, value of the current nest and mate may decrease and males may focus on their next breeding attempt, consequently, their efforts at their current nest decreases.

We found no support for the hypothesis that repertoire size bears information for females seeking extra-pair copulations (Searcy, 1992). However, other qualities of male song, for instance song rate or longevity may be important when deciding on whether to engage in extra-pair copulations with a certain male or not. Another alternative explanation is that females did not show interest for extra-pair copulations in the proximity of their social mate, rather they actively seek out males at different nest sites. Although we found no response by resident females to song playbacks at their nest, the elaborate song of males is likely involved in female choice in penduline tits.

In conclusion, acoustic signals seem to play important role in the intrasexual communication of male penduline tits. Our results suggest that repertoire size is an honest signal, by which resident males indicate their ability and willingness to defend their nest site against intruders. Further studies may reveal whether male song in penduline tits has dual function, i.e. whether it is involved also in intersexual communication.

VI SEX DIFFERENCES IN CARE PROVISIONING DO NOT EXPLAIN FEMALE-BIASED UNIPARENTAL CARE IN THE PENDULINE TIT *REMIZ PENDULINUS*

Abstract

Parental care is costly, thus evolutionary theory predicts each parent should shift care provisioning to its mate ('sexual conflict over care'). We investigated sexual conflict over care in a small passerine bird, the Eurasian penduline tit that exhibits one of the most complex avian breeding systems: sequential polygamy by both sexes, and uniparental care whereby either of the parents deserts during egg-laying, and its mate incubates the eggs and rears the chicks to independence. In Southern Hungary, female penduline tits cared more often for the clutch than males (47 % *versus* 14 %, respectively, of 534 clutches; binomial test using 0.5 expectation, $P < 0.001$), whereas 39 % of clutches were deserted by both parents. Female-cared clutches were larger (5.8 ± 0.1 (SE) eggs, 164 clutches) than those cared for by males (3.6 ± 0.2 eggs, 50 clutches; *t* test: $t_{212} = 10.235$, $P < 0.001$). We hypothesised that female penduline tits provide better care than males, and this selects for female-biased care (parental quality hypothesis). Using video recordings and observations of caring parents in six years, we show that neither incubation behaviour nor brood-feeding rates were different between males and females after controlling for clutch size, egg-laying date and ambient weather. Consistent with these results, offspring survival and nestling size did not differ between male-cared and female-cared clutches; our results therefore do not support the parental quality hypothesis. We propose alternative explanations to explain more frequent care by females.

Ákos Pogány, René E van Dijk, Péter Horváth, Tamás Székely. *Submitted to Animal Behaviour*

Introduction

Sexual conflict theory suggests males and females should adopt strategies that maximize their own reproductive success, regardless of the interest of their mate (Lessells, 1999; Parker, 1979). Since the interests of males and females over reproduction are often different, conflict over parental care may emerge such that each parent prefers the other to work harder in provisioning the young (Arnqvist and Rowe, 2005; Houston et al., 2005; Lessells, 1999). One of the extreme outcomes of parental conflict (or post-zygotic sexual conflict, Royle et al., 2002) is offspring desertion: by deserting its offspring and mate, a parent leaves the burden of full care to its partner, whereas he/she is exempt from the costs of care and may seek new partners (Houston et al., 2005; van Dijk and Székely, 2008).

In animals with uniparental care, the female more frequently provides care for the young, although exceptions are abundant (Berglund and Rosenqvist, 2003; Clutton-Brock, 1991; Reynolds et al., 2002; Székely et al., 1996; Tallamy, 2001). Two fundamental arguments have been proposed to explain why female care is more likely than male care. First, the variance in reproductive success is often higher for males, so by caring for the young the most successful males in a population would sacrifice higher reproductive success than the most successful females (Kokko and Jennions, 2003; Queller, 1997). Thus females are expected to evolve toward enhancing the efficacy of care provisioning (parental quality hypothesis, Eckert and Weatherhead, 1987; Erckmann, 1983), whereas males are expected to evolve toward being better competitors for mates. Second, in internally-fertilizing organisms males may be cuckolded, so the best interest of males is often to secure and fertilise many females instead of investing into parental care (Kokko and Jennions, 2008; Queller, 1997; Trivers, 1972; Westneat and Stewart, 2003). Consequently, both preceding arguments suggest that female-only care should evolve more likely in a population than male-only care (Kokko and Jennions, 2003; McNamara et al., 2002).

Species with natural variation in their breeding systems offer excellent opportunities to investigate these evolutionary processes. Here we investigate the parental quality hypothesis in Eurasian penduline tits using a 6-year dataset from Southern Hungary. Firstly, we predicted that females should provide better care than males, for instance, they should allocate more time to incubation and feed the young more frequently. Secondly, we reasoned that more parental effort should lead to higher offspring survival and better nourishment in female-cared broods. Thirdly, since male-cared clutches are smaller than female-cared ones (Persson and Öhrström, 1989), we also predicted that males

are less able to care for large clutches than females, therefore offspring survival and nestling size should decline steeper with increasing clutch size in male-cared nests than in female-cared ones.

Methods

Fieldwork

We investigated penduline tits at Fehértó in 2002 – 2007 between April and July. Between 2005 and 2007 male-cared (MC) and female-cared (FC) clutches were recorded during incubation and feeding (see *Chapter 2* of Appendix for supplementary information on sample sizes). For incubation analysis, 29 nests (9 MC and 20 FC) were video recorded once for approximately three hours (2.83 ± 0.07 h, mean ± SE) between the 7^{th} and 10^{th} day of incubation – at least a day after trapping when nest-trap was used. Sample size for MC is small, because fewer nests are cared for by males than by females (see Results). Recording started at a randomly-selected time between 7 a.m. and 5 p.m. We used a Sony DCR-TR7000 Hi8 and a Panasonic NV-DS28EG mini DV digital recorder mounted on a tripod. The camera was placed approximately 15 m from the nest, so that it did not disturb the incubating parent. The position of the camera was chosen to provide the best image of the nest entrance.

Feeding behaviour by the parent was observed (or recorded) once at 30 nests (10 MC and 20 FC, *Chapter 2* of Appendix) for approximately two and a half hours (2.58 ± 0.11 h, mean ± SE) on the 10^{th} day of brood care, starting randomly between 7 a.m. and 5 p.m. In 2005 and 2006 24 nests (7 MC and 17 FC) were observed by field assistants using a hide more than 15 m from the nest, and they recorded the number of nest visits. In 2007, six nests (three MC and three FC) were video-recorded using the same equipment and field protocol as for incubation, and feeding frequency was quantified using the video recordings. Uniparental care by the male or the female was confirmed at all nests involved in incubation or feeding observations and recordings; only one parent (male or female, respectively) was recorded at these nests.

We collected offspring survival data between 2002 and 2007 at 150 nests (33 MC and 117 FC, *Chapter 2* of Appendix). At each nest we counted the number of eggs ('clutch size') on the 8^{th} day of incubation, and the number of nestlings on the 10^{th} day after hatching of the first chick. Using clutch size and the number of nestlings, we calculated offspring survival as the proportion of eggs that produced 10-day old nestlings.

Between 2002 and 2004 we ringed offspring in 90 nests (17 MC and 73 FC, *Chapter 2* of Appendix). We also observed clutch size, brood size at hatching and number of chicks 10 days after hatching. Body mass and tarsus length of 10-day old chicks were measured to estimate offspring size. The different sample sizes between years are due to missing data: for each analysis we included the maximum number of nests to improve statistical power.

Data processing and statistical analyses

From the incubation video footage we recorded the parent's departure from, and arrival at, the nest. We defined departure and arrival as when the parent left the nest chamber and completely entered the nest, respectively, and incubation time was the percentage of time spent inside the nest.

From feeding observations and video recordings, we calculated feeding rates for the brood. Nest visits without food delivery were rare (1 or 2 out of 80 nest visits), we therefore used the total number of nest visits as a measure of feeding behaviour. Feeding rates increased with brood size, therefore we calculated feeding frequency per chick per hour.

Ambient weather is likely to influence incubation and feeding behaviour (Conway and Martin, 2000), so we controlled for the possible confounding effects of ambient temperature and wind speed on parental behaviour using data from a meteorological station (46º22'N, 20º06'E, about 5 km from Fehértó). The meteorological station's data provided a good approximation to the local weather as indicated by a high correlation between the station's data and the temperature readings we took in Fehértó on 24 days using a Tinytag Plus data logger (Gemini Data Loggers, UK Ltd., Pearson correlation, $r_p = 0.949$, $P < 0.001$, $N = 24$ days).

The effects of caring sex, clutch or brood size, ambient temperature and wind speed on incubation time (arcsine transformed) and feeding rate were investigated using General Linear Models (GLMs). In initial models the year of study, time of the day, and egg-laying dates were also included. The latter was calculated as the number of days from 1 April until the laying date of the first egg.

The body mass and tarsus length of chicks were averaged for each brood, and we used either mean body mass (or mean tarsus length) as a dependent variable to investigate the effect of parental sex on offspring size using GLMs. Models of offspring size included the following explanatory variables: caring sex, offspring age (in days), clutch size, number of chicks at hatching and brood size at 10 days after hatching. The effects of caring

sex and initial clutch size on offspring survival were investigated using binomial Generalized Linear Models.

Statistical analyses were carried out in R 2.6.1. (R Development Core Team 2005, Vienna, Austria). Non-significant terms were eliminated in a stepwise manner until the final models were reached. We also tested for statistical interactions between caring sex and the remaining explanatory variables in the models, since these would indicate differential effects of caring sex in regard to specific explanatory variables. We provide statistics for excluded variables before their exclusion from the model. Mean ± SE and two-tailed probabilities are given, and we rejected the null-hypotheses at $P < 0.05$.

Results

Patterns of care and clutch size

Out of 534 clutches, the female cared for 47.4 % of clutches, the male for 13.5 % of clutches, and 39.1 % of clutches were deserted by both parents. Biparental care of clutch (or brood) has never been observed in our population. Female-only care was more common than male-only care (binomial test using 325 uniparental clutches and 0.5 expectation, $P < 0.001$).

Females cared for larger clutches (5.76 ± 0.10 eggs, $N = 164$ clutches) than males (3.56 ± 0.19 eggs, $N = 50$ clutches; t test: $t_{212} = 10.235$, $P < 0.001$, Figure VI.1a). Clutch size declined over the breeding season (Figure VI.1b), and male-cared clutches were initiated later than female-cared ones (Persson and Öhrström, 1989; Pogány et al., 2008b; Szentirmai et al., 2005a). Nevertheless, female-cared clutches remained significantly larger when egg-laying date was included in the model (Figure VI.1, Table VI.1). The non-significant interaction term between caring sex and egg-laying date suggests that the decline in clutch size was comparable between male-cared and female-cared clutches (Figure VI.1b, Table VI.1), therefore this interaction was not included in the final model.

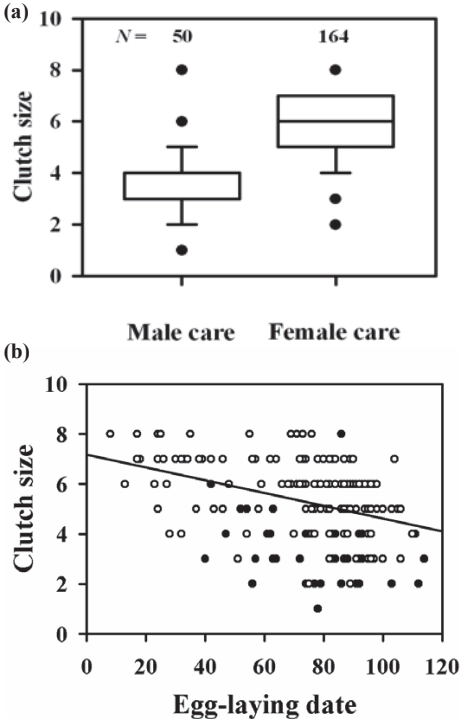

Figure VI.1. Clutch size in Eurasian penduline tits (a) in male-cared and female-cared clutches (*t* test: t_{212} = 10.235, $P < 0.001$), and (b) in relation to egg-laying date (least squares linear regression: b = -0.356, t = 5.555, $P < 0.001$). The box-plots (a) indicate the median, 10th, 25th, 75th and the 90th percentiles. The circles indicate outliers outside the 10th and 90th percentiles. Egg-laying date (b) is the number of days between the first egg laid in the clutch and 1 April. Filled symbols: male-only care, open symbols: female-only care.

Table VI.1. Clutch size (response variable) in Eurasian penduline tits. General Linear Model using 214 clutches (164 female-cared and 50 male-cared clutches). All parental sex x explanatory variable interactions were non-significant thus they were excluded (final model: $R^2 = 0.457$, $F_{7,206} = 24.761$, $P < 0.001$).

Clutch size	***F***	***DF***	***P***
Explanatory variables in the final model			
Year	6.585	5	< 0.001
Egg-laying date	48.200	1	< 0.001
Parental sex	92.200	1	< 0.001

Incubation behaviour and chick feeding

Males and females did not differ in the time they spent on incubation, and we found no difference between the sexes in response to any explanatory variables (Table VI.2). Both males and females spent more time on incubation as ambient temperature decreased (Figure VI.2a, Table VI.2), and wind speed increased (Figure VI.2b, Table VI.2); therefore, ambient weather was a good predictor of penduline tit incubation behaviour.

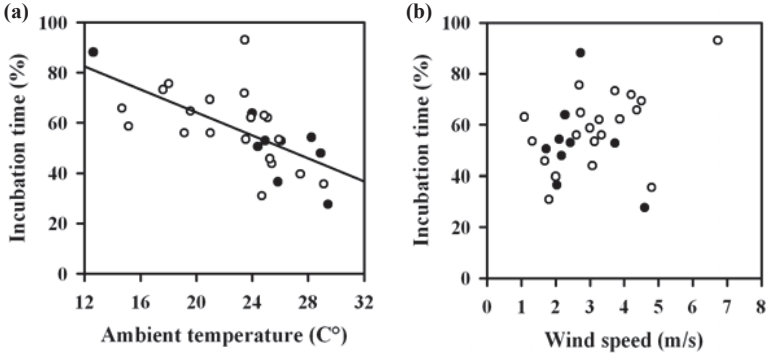

Figure VI.2. Effects of (a) ambient temperature and (b) wind speed on incubation behaviour of penduline tit parents, measured as the percentage of time inside the nest (N = 29 clutches: 9 male-cared and 20 female-cared nests; least squares linear regressions: temperature, b = -0.651, t = 4.452, P < 0.001; wind speed, b = 0.347, t = 1.924, P = 0.065). Filled symbols: male-only care, open symbols: female-only care.

Similarly, there was no difference between males and females in brood feeding rates (Table VI.2). Feeding rates decreased with brood size (Figure VI.3) and varied between years, and only these explanatory variables – not the sex of caring parent – were included in the final model (Table VI.2).

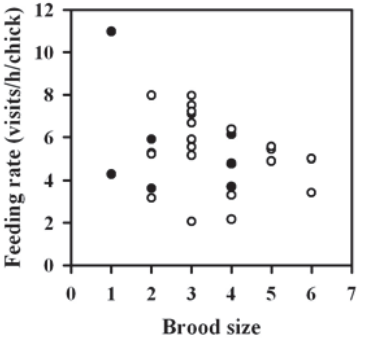

Figure VI.3. Feeding rates (nest visits per hour per chick) at penduline tit nests in relation to brood size (least squares linear regressions: b = -0.303, t = 1.680, P = 0.104). Filled symbols: male-only care, open symbols: female-only care.

Table VI.2. Incubation time (arcsine transformed proportion of time inside the nest, response variable) and feeding rate (number of nest visits per hour per chick, response variable) in penduline tits. General Linear Models using 29 clutches (9 male-cared and 20 female-cared clutches) and 30 clutches (10 male-cared and 20 female-cared clutches) for incubation and feeding, respectively. Besides the excluded variables, all parental sex x explanatory variable interaction terms were non-significant and excluded (final models: incubation time, $R^2 = 0.499$, $F_{2,26} = 12.955$, $P < 0.001$; feeding rate, $R^2 = 0.460$, $F_{3,26} = 7.394$, $P < 0.001$).

Incubation time	***F***	***DF***	***P***
Explanatory variables in the final model			
Temperature	20.790	1	< 0.001
Wind speed	5.120	1	0.032
Excluded variables			
Parental sex	0.196	1	0.662
Clutch size	0.129	1	0.723
Egg-laying date	0.663	1	0.423
Time of day	0.054	1	0.819
Year	0.256	2	0.777
Feeding rate	***F***	***DF***	***P***
Explanatory variables in the final model			
Brood size	6.509	1	0.017
Year	7.836	2	0.002
Excluded variables			
Parental sex	0.084	1	0.774
Temperature	0.375	1	0.546
Wind speed	0.404	1	0.531
Egg-laying date	0.178	1	0.676
Time of day	0.330	1	0.570

Number, survival and size of offspring

Female-cared clutches produced more nestlings (3.64 ± 0.13 chicks, $N = 117$ nests) than male-cared ones (2.56 ± 0.19 chicks, $N = 33$ nests; t test: $t_{148} = 3.645$, $P < 0.001$). However, offspring survival was not different between male-cared and female-cared clutches, after controlling for year of the study, initial clutch size and egg-laying date (Table VI.3, Figure VI.4).

Although offspring survival decreased steeper in response to initial clutch size in male-cared clutches than in female-cared ones (as shown by a weakly significant parental sex x clutch size interaction; Figure VI.4, Table VI.3), this interaction appeared to be driven by the 4-egg clutches where the male-cared offspring tended to survive better than female-cared ones (Figure VI.4).

Table VI.3. Offspring survival (proportion of eggs that produced 10-day old nestlings, response variable) in penduline tits. Binomial Generalized Linear Model using 150 nests (33 male-cared and 117 female-cared clutches). Parental sex x clutch size interaction was statistically significant, thus included in the final model.

Offspring survival	χ^2	***DF***	***P***
Explanatory variables in the final model			
Clutch size	5.295	1	0.021
Egg-laying date	9.940	1	0.002
Year	11.348	5	0.045
Parental sex	2.178	1	0.140
Parental sex x Clutch size	3.861	1	0.049

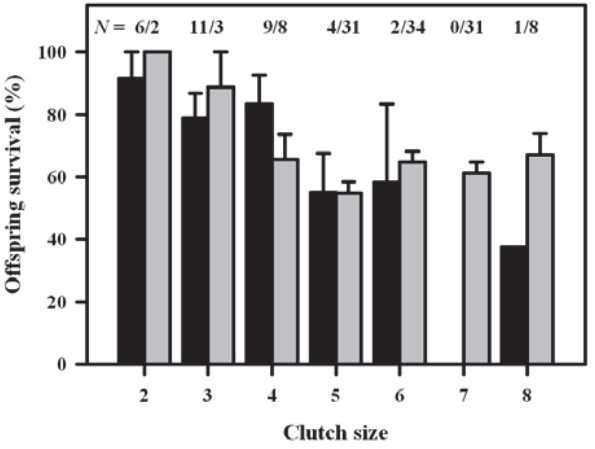

Figure VI.4. Offspring survival in relation to clutch size (least squares linear regression: b = -0.293, t = 3.725, $P < 0.001$). Offspring survival is the percentage of eggs that produced 10-day old nestlings. Filled bar: male-only care, shaded bar: female-only care. Number of clutches are given above each bar and + 1 SEM shown by error bars.

Size of nestlings was not different between male *versus* female-cared broods, although chick body mass decreased with egg-laying date and with brood size at hatching (Figure VI.5, Table VI.4). Both body mass and tarsus length increased with chick age, and were different between years (Table VI.4).

Table VI.4. Body mass and tarsus length of penduline tit chicks (response variables). General Linear Models using 90 clutches (17 male-cared and 73 female-cared clutches). All parental sex x explanatory variable interactions were non-significant and excluded (final models: body mass, $R^2 = 0.392$, $F_{5,84} = 10.831$, $P < 0.001$; tarsus length, $R^2 = 0.469$, $F_{3,86} = 25.273$, $P < 0.001$).

Offspring body mass	***F***	***DF***	***P***
Explanatory variables in the final model			
Year	8.600	2	< 0.001
Egg-laying date	5.121	1	0.026
Offspring age	19.464	1	< 0.001
Brood size at hatching	12.368	1	< 0.001
Excluded variables			
Parental sex	0.459	1	0.500
Initial clutch size	< 0.001	1	0.987
Current brood size	1.192	1	0.278
Offspring tarsus length			
Explanatory variables in the final model			
Year	29.069	2	< 0.001
Offspring age	17.683	1	< 0.001
Excluded variables			
Parental sex	0.014	1	0.906
Egg-laying date	0.068	1	0.794
Initial clutch size	0.018	1	0.893
Brood size at hatching	0.025	1	0.876
Current brood size	0.042	1	0.838

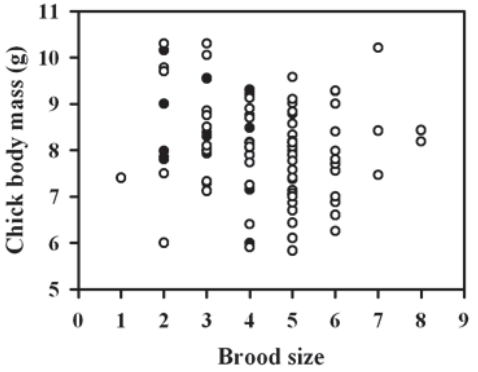

Figure VI.5. Body mass of nestlings in relation to brood size (least squares linear regression: $b = -0.164$, $t = 1.562$, $P = 0.122$). Filled symbols: male-only care, open symbols: female-only care.

Discussion

In Eurasian penduline tits, that exhibit natural variation in care-giving parent, the quality of care was not different between care by single male and single female parents: they allocated similar amount of time to incubation, and fed their young at similar frequencies. Further, we found no sex difference in survival (or size) of offspring cared for by single males or females.

Why do female penduline tits provide care more often than do males? We suggest three explanations. First, the benefit of desertion may be higher for the male than for the female. Even though in penduline tits either sex may increase its reproductive success by desertion (Szentirmai et al., 2007), the relative costs and benefits of desertion may still be different for males and females, so that the net gain may be higher for the male. To investigate this proposition, we need to extend data collection in future years, and compare the Bateman-gradients between males and females (Andersson and Iwasa, 1996; Arnold and Duvall, 1994; Bateman, 1948).

Second, even if the benefits of desertion were similar for males and females, males may be better at manipulating their partner to an outcome of sexual conflict that is more favourable for the males (Gavrilets et al., 2001). For instance, recent field observations and experiments showed that female penduline tits prefer males with large masks (Kingma et al., 2008; Pogány and Székely, 2007), and male penduline tits with a large mask deserted more often than males with a small mask (van Dijk et al. unpublished). Therefore, mask size may act as a manipulative trait by which males with large mask entice their partner to

care – perhaps by a mechanism similar to differential allocation (Burley, 1988; Sheldon, 2000). Males may also gather cues about egg-laying behaviour of their females, and this gives them the upper hand in deciding first whether care for the clutch or desert (Valera et al., 1997).

Third, males may provide less care because they are unsure about paternity in their offspring (Queller, 1997). Using molecular markers, we are currently investigating the rate of extra-pair paternity in penduline tits; Schleicher et al. (1997) found 6.9 % of EPP whereas our unpublished data suggest slightly higher rates (16 %), and no difference between male-only and female-only clutches (Mészáros et al. in prep).

Male and female penduline tits spent comparable time on incubation, and they fed their young at similar frequencies. Incubation time did not differ although males incubated on average 40 % smaller clutches. Besides, and in contrast with other studies, the number of eggs did not influence incubation effort within either sex, suggesting that incubation time in penduline tits is not adjusted to clutch size (Dobbs et al., 2006; Haftorn and Reinertsen, 1985). A possible explanation for this discrepancy is that penduline tits have well-insulated nests (Szentirmai et al., 2005b) compared to many passerine birds that nest in tree-holes. Moreover, penduline tits gain body mass during incubation, suggesting that incubation does not have an excessive energetic cost (Bleeker et al., 2005). Similar to females, male penduline tits also develop a brood patch during incubation (Cramp et al., 1993), and this further decreases the opportunity for parental quality differences to emerge during incubation. Brood size, in contrast with incubation time, constrained per chick feeding frequency, however the decrease was comparable between male-cared and female-cared broods.

We found no sex differences in offspring survival nor in chick size and mass, although female-cared clutches still produced more offspring as a result of sex differences in clutch size. Survival decreased with advance of the breeding season, which may reflect seasonal changes in food availability. An alternative explanation for the seasonal variation is provided by the optimal annual routine hypothesis (McNamara and Houston, 2008), as parents may trade-off parental efforts in favour of their own reserves before the autumn migration starts. Further in line with the annual routine hypothesis, clutch size also decreased with advance of the breeding season, as in many other bird species (Rowe et al., 1994). Sexes differed in offspring survival in response to initial clutch size, however, the steeper decrease for males was the result of higher survival in male-cared clutches containing four eggs, rather than lower survival in large male-cared clutches.

Although our results are consistent between different measures including both indirect and direct measures of male and female parental quality, it is possible that some implications of the caring sex might have remained hidden. For instance, quality of food (size or type of prey) given to offspring might have been different between male-only and female-only nests. As a consequence, recruitment (or lifetime reproductive success) of the offspring could be different between male-cared and female-cared broods.

We suggest that males care for smaller clutches than females because care provisioning is one of their viable options once their female deserted. Desertion usually takes place on day 3 or 4 of egg-laying (R. E. van Dijk, unpublished data). If the male deserts, the female usually lays a few more eggs, resulting in a large clutch (up to 8 eggs). However, if the female deserts, the male either deserts as response, or if he decide to care he ends up with incubating a small clutch (3-4 eggs). There is clearly a need for an experimental mate-removal during egg-laying and/or clutch-size manipulation to test whether small clutch size in male-cared nests is a consequence of female desertion. Furthermore, it remains to be explored how the long-term costs of care may vary between males and females; if care reduces male survival more than that of females, one would expect to see female-biased parental care in penduline tits.

In conclusion, our results are not consistent with the argument that the female-biased uniparental care in Eurasian penduline tits is due to females providing better care than males. Further studies may reveal whether the observed female-biased care is driven by sex difference in the benefits (or costs) of offspring desertion. We conjecture that in Eurasian penduline tits either the males have higher mating opportunity (and thus a steeper Bateman gradient) than the females, or by providing care the males pay higher mortality cost in future years than do females.

VII SEXUAL CONFLICT AND CONSISTENCY OF OFFSPRING DESERTION IN EURASIAN PENDULINE TIT *REMIZ PENDULINUS*

Abstract

The trade-off between current and future parental investment is often different between males and females. This difference may lead to sexual conflict between parents over care provisioning in animals that breed with multiple mates. One of the most obvious manifestations of sexual conflict over care is offspring desertion whereby one parent deserts the young to increase its reproductive success at the expense of its mate. Offspring desertion is a wide-spread behaviour, and its frequency often varies within populations. We studied the consistency of offspring desertion in a small passerine bird, the Eurasian penduline tit *Remiz pendulinus,* that has an extremely variable breeding system. Both males and females are sequentially polygamous, and a single parent (either the male or the female) incubates the eggs and rears the young. About 28-40 % of offspring are abandoned by both parents, and these offspring perish. Here we investigate whether the variation in offspring desertion in a population emerges either by each individual behaving consistently between different broods, or it is driven by the environment. Using a three-year dataset from Southern Hungary we show that offspring desertion by females is consistent between nests. Male desertion, however, depends on ambient environment, because all males desert their nests early in the season and some of them care late in the season. Therefore, within-population variation in parental care emerges by sexually different mechanisms; between-individual variation was responsible for the observed pattern of offspring desertion in females, whereas within-individual variation was responsible for the observed pattern in males. To our knowledge, our study is the first that investigates repeatability of offspring desertion behaviour in nature. The contrasting strategies of the sexes imply complex evolutionary trajectories in breeding behaviour of penduline tits. Our results raise an intriguing question whether the sexual difference in caring/deserting decisions explain the extreme intensity of sexual conflict in penduline tits that produces a high frequency of biparentally deserted (and thus wasted) offspring.

Ákos Pogány, István Szentirmai, Jan Komdeur, Tamás Székely, 2008. *BMC Evolutionary Biology* 8:242.

Introduction

Evolutionary interests of males and females are often different over reproduction (sexual conflict, Parker, 1979). Such difference may emerge from divergent optima over the number of matings (Arnqvist and Rowe, 2005; Hosken and Stockley, 2004; Parker, 2006; Rice, 1996), or over provisioning the offspring by the parents (Houston et al., 2005; Lessells, 1999). Since the benefit of rearing young is shared approximately equally by the biological parents (but make allowances for genomic imprinting, Efstratiadis, 1994; Haig, 1997), whereas each parent pays the cost of caring itself, the best interest of parents is often to shunt care provisioning to their mate (Houston et al., 2005; Lessells, 1999). One of the most obvious manifestations of sexual conflict between parents is offspring desertion whereby one parent leaves the burden of care provisioning to its mate (Székely et al., 1996).

Offspring desertion occurs in a variety of organisms including insects, fishes, amphibians, birds and mammals (McNamara et al., 2002; Reynolds et al., 2002; Robertson and Roitberg, 1998). Typically one sex abandons the young, for instance, in mammals it is usually the male that withholds care, whereas in majority of fishes the female does so (Reynolds et al., 2002). Desertion is beneficial for the deserting parent, since it improves his/her chances for reproduction in future, whereas it is costly for the abandoned mate in terms of time and energy spent on reproduction (Balshine-Earn, 1995; Houston et al., 2005; Roff, 2002; Steinegger and Taborsky, 2007; Wedell et al., 2006). In a handful of species, however, either the male or the female may abandon the young, and leave the provisioning of full care to its mate (McNamara et al., 2002; Székely et al., 1996). In these species, behaviour of an individual may depend on the behaviour of its mate as well as behaviour of other individuals in the population (Barta et al., 2002; Lazarus, 1990; Maynard Smith, 1977; 1982; McNamara et al., 2002). Therefore, full understanding of care and desertion patterns requires a game-theoretical analysis that includes (but not restricted to) costs and benefits and the process of interactions (van Dijk et al., 2007; Webb et al., 1999).

In any given population, variation in parental care behaviour may emerge in three ways. First, individuals may have different propensities to desert or care, and this propensity is consistent for a given individual over a breeding season, or over its lifetime. Second, each individual exhibits variable behaviour, and this variation is driven by environmental cues, such as differences in day length (i.e. time in the season), habitat quality, or operational sex ratio (the ratio of sexually receptive females and males, e.g.

Forsgren et al., 2004). Third, each individual behaves randomly. Although understanding parental decisions is fundamental for predicting breeding systems and the evolution of sex roles (Clutton-Brock, 2007; Kokko and Jennions, 2003; Maynard Smith, 1977; Queller, 1997; Trivers, 1972), it is striking that no study has yet tested the consistency of caring/deserting decisions in a natural population.

We investigated the repeatability in caring/deserting behaviour in the Eurasian penduline tit in two contexts. First, we investigated whether desertions by males and females are consistent between subsequent nests ('consistency analysis', henceforth). We prefer the term 'consistency' over 'repeatability', because in repeatability analysis the traits typically have normal distribution, so that General Linear Models (GLMs) can be used to separate within- and between-individual variations (Dingemanse et al., 2002; Lessells and Boag, 1987). Caring/deserting, however, is a binomially distributed trait and we used Monte Carlo Simulation (Manly, 2006). Second, we tested whether ambient environment influenced caring/deserting behaviour. Specifically, we tested whether individual behaviour changes over the breeding season ('trend analysis', henceforth). Since both abiotic and biotic variables (e.g. ambient temperature, day length, food availability) and the social environment (e.g. the number of potential mates) often vary over the breeding season, desertion behaviour, if it depends on some of these variables, should reflect seasonal variation.

Methods

Study site and data collection

Data was collected at Fehértó between 1 April and 19 August each year (2002-2004) that included the main breeding season.

Data processing

For each individual all nests in a given year were included in the analyses. If an individual had multiple nests from more than one year (3 out of 60 males, 1 out of 21 females), either the year with the highest number of nests was included, or in case of equal number of nests we chose a year randomly. We constructed one data set each for males and females. The same data sets were used for the analyses of both consistency and seasonal trend. In each data set, rows represented individuals and columns represented their subsequent nests. Score 1 and 0 indicated nest desertion and care, respectively.

In both male and female data sets, only individually ringed males and females were included, respectively. Female data set has smaller sample size, since females are more difficult to trap. The number of nests and the proportion of desertions are given in Table VII.1. These sample sizes are larger than those in former studies of caring/deserting behaviour (see McNamara et al., 2002).

Table VII.1. Number of nests used in randomizations for males and females (mean ± SE). Two data sets were constructed in which male and female penduline tits were analyzed separately. Desertion date of nests is the number of days since 1 April in each year. *U* and probability (*P*) of Mann-Whitney tests (early *versus* late nests for each data set) are also provided.

	Male	**Female**
No. of individuals	57	20
No. of individuals per year	19 ± 2.6	6.7 ± 1.8
No. of nests	157	53
No. of nests per year	52.3 ± 8.5	17.7 ± 5.2
No. of nests per individual	2.75 ± 0.15	2.65 ± 0.17
Deserted nests (%)	91.7	49.1
Deserted nests per year (%)	91.3 ± 2.1	50.4 ± 12.7
Desertion date (no. of nests)		
Early nests	67.9 ± 2.1 (72)	51.7 ± 5.1 (22)
Late nests	94.1 ± 1.4 (72)	87.4 ± 3.1 (23)
Mann-Whitney *U*	411	65.5
P	< 0.0001	< 0.0001

Desertion consistency analysis

For each individual we calculated the absolute differences between his/her scores for all possible comparisons between two nests. For example, if an individual had three nests (a, b, c), the differences between scores of all possible nest pairs were calculated as |a – b|, |a – c|, and |b – c|. Then for each individual the proportion (*p*) of consistent decisions between nest pairs was calculated as

p = no. of nest pairs where difference is zero / no. of all possible comparisons (1)

The mean of these proportions across individuals was taken as the critical value of test statistic (ΔC_{crit}).

Then each observation was randomly allocated into a position without replacement, thus randomization preserved all observations and the data structure. Randomization was iterated 10^4 times, and at each iteration the test statistic (ΔC) was calculated as above. Randomization was carried out by Resampling Stats for Excel (2006). Finally, the probability of ΔC larger than ΔC_{crit} was calculated (P), and we report this value.

Trend analysis

Each row in the data sets was divided into first and second half, representing nests built during early or late breeding season, respectively. Rows with an odd number of nests had the middle nest eliminated. Early *versus* late nests for a given individual correspond to early and late calendar dates of nest desertion; relative desertion dates (number of days from 1 April in each year) of early *versus* late nests differed in both male and female data sets (Table VII.1).

The mean score of early nests and late nests was calculated separately; for instance, for an individual with desertion history 1,1,0,1, the means of early and late nests were 1 and 0.5, respectively, whereas for an individual with desertion history 1,0,1,1,0, the corresponding means were 0.5 and 0.5. Then the mean score of late nests was subtracted from the mean score of early ones, and finally, the test statistic (ΔT_{crit}) was calculated as the mean of all these differences. For the two individuals in the preceding example ΔT_{crit} = (0.5 + 0) / 2 = 0.25. Therefore, positive ΔT_{crit} indicates more desertion early in the season than later, whereas a negative ΔT_{crit} indicates *vice versa.* Accordingly, values close to zero indicate no seasonal change in care pattern.

In trend analyses the randomization followed the same logic as in consistency analysis (see above), so that the mean difference (ΔT) was calculated in 10^4 iterations. We then took the probability (P) of higher (if ΔT_{crit} was positive), or lower (if ΔT_{crit} was negative) ΔT than the test statistic.

Results

Consistency of parental care

Caring/deserting behaviour of males was over-randomized ($P = 0.991$, $\Delta C_{crit} = 0.801$, $N = 57$ males); thus if a male deserted one of his nests, he was more likely to care for his next nest. Female behaviour, however, was consistent between nests ($P = 0.037$, $\Delta C_{crit} = 0.650$, $N = 20$ females).

Seasonal trend in parental care

Concordantly with the results of consistency analysis (see above), males changed their behaviour with advance of the breeding season. Males uniformly deserted early in the season although some males cared later in the season ($P < 0.0001$, $\Delta T_{crit} = 0.199$, $N = 57$ males; Figure VII.1). Female behaviour, however, did not change over the breeding season ($P = 0.148$, $\Delta T_{crit} = -0.150$, $N = 20$ females; Figure VII.1).

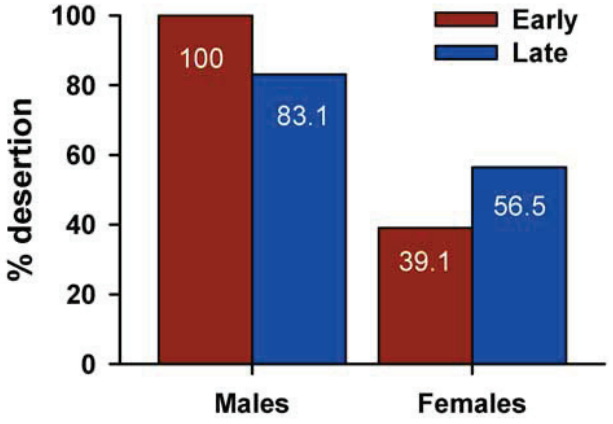

Figure VII.1. Nest desertion by male and female Eurasian penduline tits. Males desert their nests early in the season, and some of them care for their late ones ($P < 0.001$, $\chi^2 = 13.075$, $N = 146$ nests). Female behaviour is not different between early and late nests ($P = 0.767$, $\chi^2 = 0.088$, $N = 46$ nests).

Discussion

We revealed sexual differences between parental care decisions of male and female penduline tits. Our findings are in line with recent studies of repeatability and genetic differences of parental behaviours (Kölliker and Richner, 2001; Lock et al., 2004; MacColl and Hatchwell, 2003a; Potti et al., 1999; Schwagmeyer and Mock, 2003), and a further step to understand parental care decisions and the evolution of breeding systems in nature.

Our main results are that female penduline tits are consistent in their desertion behaviour, and male behaviour is predicted by ambient environment, in terms of early *versus* late season. Female behaviour varied little between subsequent nests, and they either cared or deserted consistently regardless of time in the season. We propose three explanations for this pattern. First, female penduline tits may vary in some traits linked to mating success, and this, in turn, would affect their care decisions. For instance, attractive (or fecund) females may desert more frequently than non-attractive (or less fecund) ones, since they are likely to remate sooner. To investigate this proposition, further work focusing on female attractiveness, fecundity and male preference is needed. Second, energy demands of various stages of reproduction may be different, and this predicts state-dependent parental decisions (Barta et al., 2002; Freeman-Gallant and Rothstein, 1999). In line with the latter suggestion, weather conditions have frequently been reported to predict offspring desertion in various species (Frere et al., 1998; Simeone et al., 2002; Wiggins et al., 1994), besides, in a recent study Bleeker et al. (2005) found that offspring desertion is influenced by body condition in penduline tits. Therefore, it is possible that body condition of female penduline tits changes slower than that of males, and this results in consistent parental behaviour in females while not in males. Third, consistent parental decisions of females may be the result of fixed genetic effects and/or imprinting in parental behaviour (Freeman-Gallant and Rothstein, 1999; Kölliker and Richner, 2001; MacColl and Hatchwell, 2003b). For instance, by crossing two types of sticklebacks *Gasterosteus aculeatus* with a different propensity to care, Blouw (1996) demonstrated that parental behaviour is heritable in laboratory circumstances. Testing heritability of caring/deserting in penduline tits, however, is challenging in nature, because offspring recruitment is low (6.0 % for males, 7.2 % for females, van Dijk et al. unpublished data)**.**

In contrast to females, parental behaviour of male penduline tits depends upon the timing in the season. We suggest this seasonal trend reflects changes in circulating hormonal levels, or seasonal variation in the sensitivity of the receptors of breeding-related hormones (Adkins-Regan, 2005). Studies with passerine birds show that individuals

breeding early in the season have higher testosterone levels than those breeding later (Adkins-Regan, 2005; Wingfield et al., 1990). Testosterone level is a key component in the trade-off between male mating effort and parental care, because high testosterone levels stimulate sexual behaviour (such as male-male competition or nest guarding), whereas it suppresses paternal care (Adkins-Regan, 2005; Ketterson et al., 1992; Peters et al., 2002; Van Roo et al., 2003; Wingfield et al., 1990). Testosterone also plays a role in the development of ornaments (Andersson, 1994; Peters et al., 2000). Consistently with these studies, male penduline tits (but not females) moult late in the season (SA Kingma, personal observation) when their testosterone level is presumably low (Wingfield et al., 1990). Therefore, timing of moulting corresponds to male care, thus seasonal change in testosterone levels seems a promising candidate for explaining the change in male parental care (Badyaev and Duckworth, 2005).

Different physiology of male and female penduline tits may contribute to the different individual strategies we showed here. Female penduline tits continue producing eggs throughout the breeding season which is unusually long, approximately 3.5 months in Hungary. If sexual hormones (e.g. prolactin) are associated with egg-laying, then these may maintain consistent behaviour throughout the season. Males, however, may have high testosterone levels early in the season that helps them to acquire mates, and as the breeding season progresses, their testosterone level may gradually decline. Coupled this with the declining number of females that are available (since most females are tied up with caring in the population), the propensity of males may change from desertion to provide care. In order to test these propositions, we need to investigate the physiological mechanisms responsible for desertion, and/or manipulate circulating hormone levels.

How are the different strategies maintained in a population? We propose two explanations for the existence of different male and female strategies. First, the seasonal trend in males, and the consistent behaviour in females may be an optimal pair of strategies. For a male, deserting early in the season is beneficial, since if his female is a 'caring' type his offspring will be catered for, whereas if his female is a 'deserting' type she may carry his sperm and fertilize eggs in her new clutch. Late in the season, however, both of these benefits of desertion diminish for the male. Currently we are testing this proposition by genotyping chicks and adults (Mészáros et al. in prep). From the female perspective, deserting early in the season looks like a costly strategy that may be balanced out by the benefit of deserting late in the season – when males are more likely to care. For females of the caring type, these costs and benefits may be reversed over the breeding

season: they benefit early in the season but pay a cost later. Whilst these arguments have their intuitive appeal, a proper understanding of the penduline tit breeding system requires a full game-theoretic model (van Dijk et al. in prep).

Second, the observed strategies may not be optimal, and the low breeding success reduces population viability. Fully fixed behavioural strategies (care/desert) would not be stable in the population, because the other sex was to exploit the fixed strategy due to sexual conflict. The high frequency of biparentally deserted nests in different European populations, however, suggests that the reproductive success of these populations is not at the maximum. Biparental desertions can be viewed as 'mistakes', since each sex assumes the other sex will care for the clutch, whereas in reality it may have already deserted. We are currently pursuing the latter proposition by analyzing the behaviour of males and females immediately preceding desertion (van Dijk et al, in prep). Biparental desertion occurs during egg-laying, and it implies that the male wastes his energy and time (often, weeks) building a sophisticated nest, and then the female wastes her effort on producing the clutch of up to five eggs. Our analyses suggest that the high frequency of biparental desertion emerge when the population consists of many females from the 'deserting' phenotype, and it is early in the season so that the males also desert. Further studies by monitoring penduline tits' population dynamics may reveal whether immigration/emigration of females with different tactics contribute to the observed patterns of offspring desertion.

Our results contribute to the different repeatabilities of male and female parental behaviour reported in other studies. Potti et al. (1999) showed that female pied flycatchers *Ficedula hypoleuca* spend repeatable amount of energy on parental care between breeding seasons, whereas the energy expenditure of males was not repeatable. Schwagmeyer and Mock (2003) and Nakagawa et al. (2007) reported food provisioning levels to be repeatable in male house sparrows *Passer domesticus*, but not in females. However, MacColl and Hatchwell (2003a) found both male and female feeding rates of long-tailed tits *Aegithalos caudatus* were repeatable. In addition, a recent study by Charmantier et al. (2007) showed high heritability in cooperative behaviour in male Western bluebirds *Sialia mexicana*. These studies together with our findings suggest that individuals of one sex may be more variable in their parental care, thus sex differences may emerge over the repeatability/flexibility of parental care.

Establishing the repeatability (or heritability) of behaviour does not negate the influences of environment on parental behaviour. For instance, food availabilities,

predation, and operational sex ratio may all be involved influencing care provisioning (reviewed by Balshine-Earn et al., 2002; Clutton-Brock, 1991; Houston et al., 2005). In addition to these ecological traits the behavioural interactions may also influence conflict resolution. Recently we showed that at biparentally deserted nests the male and female desert on the same day (van Dijk et al., 2007). The latter result raises the intriguing possibility that desertions may not be independent by males and females (McNamara et al., 2002). To explore this proposition, one needs larger sample sizes for powerful statistical analyses that can distinguish between competing theoretical scenarios. We suspect that ecological variables and genetic (or learnt) predispositions may interact, and this further underlies the significance of larger datasets than those we currently have, and the need of experimental manipulations.

In conclusion, we analyzed within-population variation in offspring desertion in a small passerine bird that exhibits one of the most complex parental care systems in birds. We showed that female penduline tits have consistent parental decisions regardless of time in the breeding season, whereas male behaviour is largely driven by timing in the season. Therefore, within-population variation in parental care emerges differently for males and females, since variation in female behaviour at population level mainly emerges by between-individual, whereas variation in male behaviour is mainly due to within-individual variation. These contrasting strategies suggest complex evolutionary trajectories in breeding behaviour of species with variable breeding system.

VIII SEXUAL CONFLICT PREDICTS MORPHOLOGY AND BEHAVIOUR IN TWO SPECIES OF PENDULINE TITS

Abstract

The evolutionary interests of males and females rarely coincide over reproduction (sexual conflict), and these conflicting interests influence morphology, behaviour and speciation of various organisms. Here we focus on two closely-related, small passerine birds: the Eurasian penduline tit (EPT) that has a highly polygamous breeding system with sexually antagonistic interests over parental care, and the socially monogamous Cape penduline tit (CPT). We derived four *a priori* predictions from sexual conflict theory, and tested these predictions using data collected in Central Europe (EPT) and South Africa (CPT). Firstly, we predicted that EPTs exhibit more sexually dimorphic plumage than CPTs due to more intense sexual selection. Secondly, more attractive EPT males should provide less care than dull males. Thirdly, male EPTs should exhibit more complex songs than CPT males. Finally, intense sexual conflict in EPT should lead to low nest attendance as an indication of sexually antagonistic interests, whereas we expected more cooperation between parents in CPT. Consistent with our predictions, EPTs exhibited greater sexual dimorphism in plumage and more complex song than CPTs, and more attractive EPT males provided less care than dull ones. Finally, EPT parents attended the nest less frequently and less simultaneously than CPT parents. These results provide support for sexual conflict theory: sexual selection is more intense in EPT than in CPT, and EPT parents appear to avoid each other at the nest. These results are consistent with the notion that EPTs attempt to force their partner to work harder.

René E van Dijk, Ákos Pogány, Penn Lloyd, Jan Komdeur, Tamás Székely. *Submitted to Behavioral Ecology*

Introduction

Reproduction has long been viewed as a cooperative exercise between male and female partners. Yet, the evolutionary interests of males and females are often different (sexual conflict, Parker, 1979). Only in the rare case of semelparity, or when there is full and lifelong monogamy of the pair members will the optimum amount of care provided be even for both parents (Lessells, 2006; van Dijk and Székely, 2008). Only recently, however, have researchers started to explore the implications of sexual conflict on speciation, breeding systems, and evolution of various life-history traits (Arnqvist and Rowe, 2005; Gavrilets et al., 2001; Hosken and Snook, 2005; Houston et al., 2005). Sexual conflict appears a potent evolutionary force that may mould morphology (Arnqvist and Rowe, 2002a) and behaviour (Chapman et al., 2003), and promote speciation (Arnqvist et al., 2000). For instance, dung fly *Sepsis cynipsea* populations undergoing more intense sexual conflict behaviourally diverged to a greater extent than flies under more relaxed conflict, resulting in different levels of reproductive isolation (Martin and Hosken, 2003). As another example, extra-pair copulations in monogamous passerines have been suggested to result from sexually antagonistic evolution. The negative selection for direct benefits from extra-pair copulations for females appears to be greater than the positive selection for indirect benefits, which supports the idea that extra-pair copulations reflect pre-zygotic sexual conflict (Arnqvist and Kirkpatrick, 2005).

Conflicts between parents over care (post-zygotic sexual conflict, Royle et al., 2002) emerge *via* a trade-off between parental effort and alternative mating opportunities for each individual parent. As a result, each parent may try to avoid the costs of care and shift those costs to the partner (Houston et al., 2005; Lessells, 1999). This may happen through a continuous adjustment of parental effort in response to the mate's current effort (best response rule, Houston and Davies, 1985), or through a discrete decision to either care for the offspring or to desert the partner and offspring (Maynard Smith, 1977). The conflict over care typically occurs when there is an opportunity to reduce parental contribution. For example, a parent may desert the brood when one parent is sufficient to successfully raise the offspring (Bart and Tornes, 1989; Székely et al., 1996). This may occur when resources are plentiful (Beissinger and Snyder, 1987) or when offspring require little care, as is often the case with precocial young (Olson et al., 2008; Wisenden, 1994). By deserting, an individual gains the opportunity to find a new mate and breed again, thereby enhancing its reproductive success (Pilastro et al., 2001; Szentirmai et al., 2007; van Dijk and Székely, 2008; but see Grüter and Taborsky, 2005). As such, sexual

conflict over care likely promotes more polygamous breeding systems (Davies, 1989; Magrath and Komdeur, 2003; McNamara et al., 2000; Székely et al., 2006). With increasing levels of polygamy the variance in reproductive success increases. Thus, more polygamous breeding systems are associated to more intense sexual selection than monogamous systems (Björklund, 1990; Gonzalez-Voyer et al., 2008; Pérez-Barbería et al., 2002; Wiklund and Forsberg, 1991). Subsequently, sexual selection is expected to act stronger in species exhibiting intense conflict than in species undergoing weak levels of conflict.

Here we test *a priori* predictions of sexual conflict theory about the impact of sexual conflict on morphology and behaviour by comparing two closely related species of penduline tits (Alström et al., 2006; Gill et al., 2005; R. E. van Dijk, M. Irestedt, P. G. P. Ericson, T. Székely, unpublished data): the sequentially polygynandrous Eurasian penduline tit ('EPT' henceforth) and the socially monogamous Cape penduline tit ('CPT' henceforth). This will elucidate the potential impact sexual conflict may have on the evolution of traits, which may eventually facilitate speciation.

Firstly, given that EPT is the more polygamous species, and thus likely experiences a larger variance in reproductive success, we predict more intense sexual selection in EPT than in the socially monogamous CPT. This is expected to result in a stronger sexual plumage dimorphism and more complex song in EPT than in CPT.

Secondly, we test the prediction that males with a large mask, which signals male attractiveness (Kingma et al., 2008; Pogány and Székely, 2007), desert the nest at a higher frequency than less attractive males, thus imposing the costs of parental care on their partner. We expected more attractive males to desert more frequently than less attractive males, because attractive males are presumably more successful at finding a new mate after desertion. As such, attractive males would benefit from desertion, whereas the females face the costs of care, and may gain a relatively lower reproductive success (Szentirmai et al., 2007).

Finally, following predictions from sexual conflict theory (e.g. Houston et al., 2005), we expect that in EPT, in which nest desertion is common, parents will try to shift the costs of parental care to the partner. Specifically, we test the prediction that in EPT the pair is expected to attend the nest less synchronously during the egg-laying phase than in CPT. Thus EPT parents are expected to shift the costs of nest attendance to the partner and therefore they will not show up at the nest until the partner has left to, for instance, forage or collect nest material. Alternatively, rather than actively avoiding each other at the nest,

EPT partners may also take over the work from each other. This would also result in less time spent together at the nest, albeit that this should be interpreted as a more cooperative behaviour as opposed to avoidance due to conflict. To distinguish between cooperation and conflict in nest attendance, one would expect that in EPT both parents attend the nest less frequently (total frequency of individual and joint attendance) than in CPT (see Royle et al., 2002).

Methods

Study sites and data collection

We studied EPTs between April and August in five consecutive breeding seasons (2003-2007) in Fehértó, Hungary where approximately 60-90 males and 45-50 females bred each year. The number of nests per year varied from a minimum of 158 in 2005 and 2006 to a maximum of 183 in 2003. CPTs were investigated in September 2006 and 2007 at Koeberg Nature Reserve, South Africa. We studied eight and six breeding pairs in 2006 and 2007 respectively, monitoring 10 and 11 nests (the discrepancy between number of breeding pairs and number of nests can be attributed to the fact that some nests are abandoned by the male in an early stage of nest-building). The low number of monitored nests in CPT compared to EPT is due to the lower population density in CPT, as large territories are used by family groups (Dean, 2005). Both species build similar, domed nests, initiated by the male. In EPT males are unpaired, whereas most CPT males are paired at the onset of building. The nest is finished and maintained jointly by both male and female after pair formation in both species. The egg-laying phase is initiated at a similar stage of nest-building, i.e. when the parents start building the entrance tube to the nest.

We searched both study areas for nest-building penduline tits, and visited each nest about every other day to determine which parent attended the nest (van Dijk et al., 2007). At each EPT nest we recorded the date of pair formation. We considered a male to be mated as soon as the pair was seen copulating near the nest or when male and female were seen to build the nest together. For time in season we used a date format as the number of days since 1 April in each year. We trapped penduline tits using mist net, song playback and dummy (in EPT) and hair-net (in CPT). Birds were ringed, morphometric measurements and digital photos were taken of each side of the bird's head to measure mask size.

The song of 16 male EPTs was recorded in 2006 for 127.5 ± 48.4 min (mean ± SD) at a randomly selected time of day between 6:28 a.m. and 5:50 p.m. (CET), using a Marantz PMD 660 portable digital recorder with a Sennheiser ME66 directional microphone. From these recordings a total of 2229 syllables were analyzed. Adding all recordings from all males together, the total number of different syllables sung in the population ('repertoire size') in EPT did not increase after 46 % of the total time of recordings (2100 min). Additionally, after the first 52 % of recorded syllables (i.e. the first hour of recording from all 16 males) we obtained 14 out of the 16 different syllables we recorded in total (i.e. 88 %). All different syllables sung by an individual male were obtained after 71 ± 24 % of the total number of syllables recorded per individual. Although this suggests we may have underestimated the repertoire size for some individual males to some minor extent, we expect that this will not have negatively affected our results at the species level, but may only make them more conservative (see Results). Using the same equipment as for EPT, we recorded the song of 9 CPT males (recording time 220.9 ± 94.3 min), which altogether sang in total 1918 syllables. All recordings for CPT were made during the morning (6:20 a.m. – 11:30 p.m. UTC). For both species, in the analyses we only included song recorded from males that had already acquired a partner. We did not find any variation in the number of different syllables sung by CPT (see Results), so we are confident that we obtained the full repertoire size for CPT. Sonograms of the recordings were created and analyzed using Audacity v. 1.2.6 and Avisoft-SASLab Light v. 3.74 softwares.

To investigate patterns of nest attendance during nest-building, which continues through the laying period, we filmed nests using a time-lapse video camera (Sony digital handycam, DCR-HC44E) storing one frame every five seconds. Nest attendance by CPT parents was recorded on the second and third day of egg-laying (547 ± 82 min per day, N = 7 pairs). Since we were not always able to determine the first day of egg-laying in EPT, we also included recordings from the period after pair formation but before egg-laying for EPT (329 ± 184 min per day, N = 21 pairs), i.e. a more extended period than for CPT. The period before egg-laying involves more nest-building than maintenance. We anticipate that this has not affected our results, since one would expect the parents to spend more time at the nest during nest-building than during nest maintenance, which is opposite to the pattern we predicted and found (see Results). Recordings were analyzed frame by frame using MATLAB v. 6.5, coding nest attendance (i.e. presence of bird on or inside the nest) as: (i) male-only, (ii) female-only, (iii) joint nest attendance by male and female, or (iv) both parents absent.

To distinguish between the sexes and intruders we used differences in plumage (Cramp et al., 1993; Kingma et al., 2008), behaviour (e.g. females are more often and for longer periods inside the nest than males, intruders are often on the outside of the nest and build very little), and colour rings. When we were not able to identify an individual attending the nest, we excluded all recordings between this frame and the next frame at which an individual bird could be recognized from the analyses. As nest desertion takes place during egg-laying in EPT, we took into account only the mated period to avoid a bias towards absence of any of the parents at the nest.

Data analyses

We used a binary logistic regression model with backward elimination to predict male care strategy (care/desert) at the first clutch of a male in response to the size of the male's mask in EPT. The initial model included year as a categorical covariate and mating date as a continuous covariate. Neither covariate contributed significantly to the model ($P > 0.255$), so both were removed from the final model. The final model provided an adequate fit to the data (Hosmer-Lemeshow goodness-of-fit; $\chi^2 = 10.289$, $DF = 8$, $P = 0.245$), and also a similar model testing whether female care could be predicted based on male mask size provided an adequate fit ($\chi^2 = 6.400$, $DF = 8$, $P = 0.603$).

All CPTs videotaped were colour ringed. The analyses for nest attendance by CPTs included one male that was recorded at two different nests, however, these two nests were in two different years and the female was different at each nest, so we consider the effect of pseudo-replication to be minimal. Out of the 21 EPT nests filmed, one male and 18 females were not colour ringed. Returning rates across years of individuals are low (5 % for males, 2 % for females, van Dijk et al., 2008), therefore it is unlikely that we observed the same unringed individuals in both years. Additionally, of the eight unringed females in 2006 and the ten in 2007, three and six bred simultaneously, respectively, and we can thus be certain that these are different individuals. For the remaining nine females in 2006 and 2007 we cannot exclude the possibility of pseudo-replication, although we suspect it is unlikely given (i) the size of our breeding population and (ii) that the composition of pairs was nearly always different (out of 194 pairs that produced a clutch, only six pairs remained together and produced a second clutch between 2002 and 2007). Pseudo-replication in the plumage analyses was avoided by randomly choosing one measurement per individual.

To examine the degree of synchrony in nest attendance by male and female, i.e. male and female being together at the nest simultaneously, we first calculated the time that the male and female can be expected to spend together at the nest by chance, by multiplying the total percentage nest attendance by the male, i.e. male-only attendance plus attendance by male and female together, with the total percentage nest attendance by the female. We then compared the difference between observed and expected patterns of nest attendance by both species using a General Linear Model. A General Linear Model was also used to compare the total proportion of time the parents spent at the nest, i.e. the sum of male-only, female-only and joint nest attendance, between the two species. Both initial models included year as a factor and the first day of filming as a covariate. Neither contributed significantly to either of the models ($P > 0.138$), so we excluded both from the final model. To test for an effect of the difference in day length between the two study sites (15 h 46 min ± 0 h 11 min in Budapest, Hungary *versus* 11 h 41 min ± 0 h 12 min in Cape Town, South Africa) on our results, we estimated the absolute time the parents attended the nest per day as the percentage of time spent at the nest x day length (day lengths for both study sites collected from http://www.timeanddate.com). We then compared whether the absolute time spent at the nest by both parents is different between the two species.

We provide the effect sizes (Cohen, 1988) and power analyses, and applied the asymptotic relative efficiency when estimating power of Mann-Whitney U-tests (Lehmann, 1975). If the power of the statistics was relatively low for CPT (i.e. $1\text{-}\beta \leq 0.5$), we provide the sample size that would be required to find a statistically significant difference between the two groups given Cohen's effect size d of the underlying data of CPT and power $1\text{-}\beta = 0.8$ ($N_{required}$), and the required sample size given the effect size d in EPT and power $1\text{-}\beta = 0.8$ ($N_{d,1\text{-}\beta}$). All statistical analyses were performed using SPSS 14 (SPSS Inc., USA), except for the power analyses, which were carried out using R 2.6.1 (R Development Core Team 2005, Vienna, Austria). We provide mean ± SD, and two-tailed probabilities.

Results

Plumage

In EPT, the mask of males was significantly (28 %) larger than that of females, whereas in CPT the size of the mask was not different between males and females (sex: $F = 9.881$, $P = 0.002$; species: $F = 295.358$, $P < 0.001$; interaction sex x species: $F = 10.290$, $P = 0.002$, $N = 206$ individuals; Figure VIII.1; Table VIII.1).

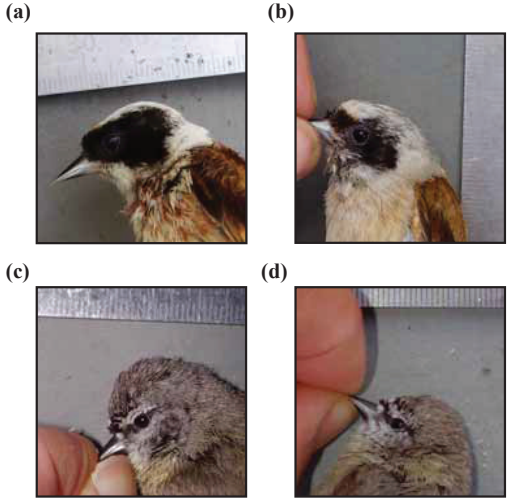

Figure VIII.1. Mask size of (a) male and (b) female Eurasian penduline tits, and (c) male and (d) female Cape penduline tits.

Table VIII.1. Mask size of males and females in EPT and CPT. *d* = Cohen's effect size, *1-β* = power. The sample size required for a statistically significant difference is provided for CPT given the effect size *d* of the underlying data of CPT and the power *1-β* set at 0.8 ($N_{required}$), and given the effect size *d* in EPT and the power *1-β* set at 0.8 ($N_{d,1-\beta}$) (see Cohen, 1988).

	Eurasian penduline tits	**Cape penduline tits**
Male mask size (cm^2)	1.29 ± 0.23 (N = 155)	0.13 ± 0.02 (N = 9)
Female mask size (cm^2)	0.93 ± 0.20 (N = 34)	0.14 ± 0.03 (N = 8)
Test statistics	t = 8.419	Z = 0.627
P	< 0.001	0.531
d	1.594	0.034
$1-\beta$	> 0.99	0.05
$N_{required}$		13581
$N_{d,1-\beta}$		8

Male EPTs with large mask were more likely to desert their first clutch than those with small and thus less attractive masks (binary logistic regression model; model effect estimate ± SE = 2.647 ± 1.226, Wald = 4.661, DF = 1, P = 0.031, N = 121 males; Figure VIII.2). Females, however, were not more likely to care for clutches when mated to males with larger masks (0.222 ± 0.786, Wald = 0.080, DF = 1, P = 0.778, N = 121 males).

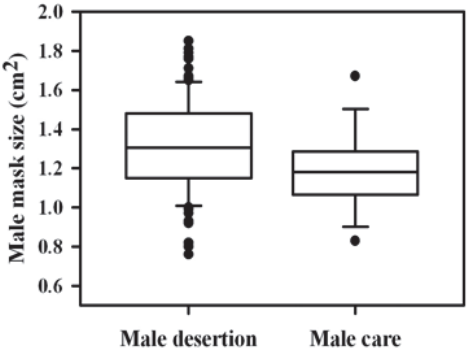

Figure VIII.2. Mask size of deserting (N = 104) and caring (N = 17) male Eurasian penduline tits. Boxplots show the median, interquartile range, outliers and extreme cases.

Song

The song output at the nest was not significantly different between EPTs and CPTs: EPT males sang 62.8 ± 32.0 (N = 16 males) syllables per hour, whereas CPT males sang 54.0 ± 42.8 (N = 9 males) syllables per hour (t = 0.581, P = 0.567, N = 25 males, d = 1.461, $1\text{-}\beta$ = 0.92). Male EPTs used 8.3 ± 2.8 different syllables (N = 16 males), whereas song was invariably mono-syllabic in CPT males (one-sample t-test; t = 10.474, P < 0.001, d = 3.029, $1\text{-}\beta$ > 0.99; Figure VIII.3).

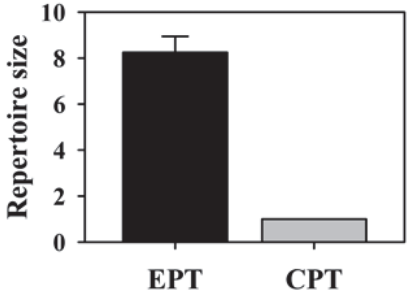

Figure VIII.3. Repertoire size, i.e. the mean number of different syllables sung by males, in Eurasian (EPT) and Cape penduline tits (CPT). Bars represent mean + 1 SEM.

Nest attendance

Eurasian and Cape penduline tit parents differed significantly in the frequency of synchronous nest attendance (Mann Whitney U-test; $Z = 3.902$, $P < 0.001$, $N = 28$ pairs, $d = 2.949$, $1\text{-}\beta > 0.99$; Figure VIII.4). This result stands when we compare the estimated absolute time spent together at the nest per day (EPT: 115 ± 162 sec, CPT: 2343 ± 864 sec; Mann Whitney U-test; $P < 0.001$). Comparing the difference between the expected time spent together at the nest by both parents (i.e. male nest attendance x female nest attendance) and the observed time across the two species, we found a significant effect of species ($F = 20.366$, $P < 0.001$, $N = 28$, $\eta^2 = 0.439$, $1\text{-}\beta = 0.99$). EPT parents spent less time together at the nest than expected by chance (0.20 % *versus* 3.23 % of time; $Z = 4.015$, $P < 0.001$, $N = 21$ EPT pairs, $d = 2.898$, $1\text{-}\beta > 0.99$; Figure VIII.4), whereas in CPT the pair spent similar time together at the nest compared to what is expected by chance (5.56 % *versus* 5.05 % of time; $Z = 0.845$, $P = 0.398$, $N = 7$ CPT pairs, $d = 0.329$, $1\text{-}\beta = 0.09$, $N_{required} = 146$, $N_{d,1\text{-}\beta} = 4$; Figure VIII.4).

EPTs attended the nest significantly less frequently during egg-laying than CPT parents (36.0 ± 9.9 % of time, $N = 21$ EPT pairs, 49.4 ± 15.7 % of time, $N = 7$ CPT pairs; $F = 7.075$, $P = 0.013$, $\eta^2 = 0.214$, $1\text{-}\beta = 0.726$).

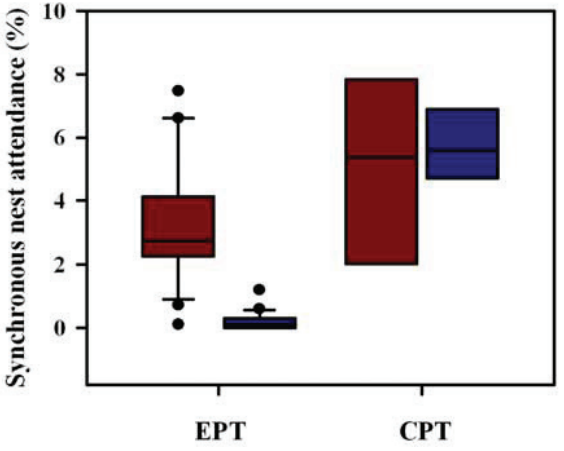

Figure VIII.4. Expected (red) and observed (blue) synchronous nest attendance by male and female Eurasian (EPT) and Cape penduline tits (CPT). The box-plots indicate the median, 10th, 25th, 75th and the 90th percentiles. Outliers (i.e. data points falling outside the 10th and 90th percentiles) are indicated by dots.

Discussion

We found support for several *a priori* predictions flowing from sexual conflict theory. Firstly, we found a clear sexual plumage dimorphism in Eurasian penduline tits but not in Cape penduline tits. Secondly, we found that EPT males had a more complex song (i.e. a larger song repertoire) than CPT males. This suggests that sexual conflict may drive the evolution of plumage dimorphism and complexity of song through increased intensity of sexual selection. We realize that the power to detect a significant difference between the sexes of CPT in mask size is low, and we acknowledge that this may have confounded this result. However, the sample size required to detect a sexual dimorphism in CPT mask size, given the effect size and power (N_{required}), is unrealistically large, yet with our sample we would have been able to detect a sex difference in mask size in CPT if it had been of a similar intensity as in EPT ($N_{d,1\text{-}\beta}$; Table VIII.1). This suggests that male and female CPTs are factually monomorphic in mask size, unlike EPTs. Additionally, we note that males and females can easily be distinguished in the field in EPT, but not in CPT, and we thus suspect that our results would not change if sample sizes in CPT would have been larger.

Thirdly, we found that EPT females mated to attractive males are more likely to be deserted by their partner. However, these females are not more likely to care for the offspring after male desertion than females mated to less attractive males. The latter could be interpreted as retaliation by the females to avoid being exploited by the males, and may partly explain the existence of biparental desertion: if an attractive male deserts, a female may still decide to desert, despite the fact the nest and eggs will be lost (see also van Dijk et al., 2007). Nevertheless, females mated to attractive males pay additional costs of reproduction compared to females mated to less attractive males: a female may obtain both direct and indirect benefits from mating with attractive males, yet these females pay the full costs of caring or, in case they desert too, all effort invested until desertion, i.e. the effort of nest-building and egg-laying, has been in vain. Additionally, indirect benefits are generally assumed not to offset the direct male imposed costs, resulting in indirect benefits being of little importance in the evolution of sexually antagonistic traits (Arnqvist and Kirkpatrick, 2005; Cameron et al., 2003; Chapman, 2006). In any case the fitness of a female is to some extent negatively affected when mated to an attractive male, as predicted under sexually antagonistic coevolution (Chapman et al., 2003; Szentirmai et al., 2007). This points out the conflict a female faces when choosing a mate: females may mate up with attractive males that may be of suboptimal quality (Chapman et al., 2003).

Our results, in addition to Kingma et al. (2008) showing that the reproductive success (number of nestlings and their survival) of females seemed to decrease with their mate's attractiveness, suggest that males may manipulate their partner *via* sexually selected traits. Kingma et al. (2008), however, found that mask size could not predict the decision over parental care. This discrepancy with our results may be explained by the fact that they used a smaller dataset, which was based on nests that occurred later in the season, when more males started to care for the offspring. The latter is possibly due to limited remating opportunities for males as more females will be either incubating or feeding the offspring (Persson and Öhrström, 1989), in which case the decision to care or desert will depend less on the male's attractiveness.

Following on from this, an alternative, additional mechanism to increasing intensity of sexual selection under sexual conflict that may potentially affect the evolution of various traits, is a process of manipulation by one partner and resistance by the other as expected to emerge from sexual conflict (Chapman et al., 2003; Lessells, 2006). This may also explain the difference in sexual dimorphism and song complexity between the two species of penduline tits. Evidence for this arms race between male and female partners derives from pre-copulatory sexual conflict where males are harmful to females during copulation (Arnqvist and Rowe, 2002a; Arnqvist and Rowe, 2002b; Crudgington and Siva-Jothy, 2000; Lessells, 2006). Males may, for instance, cause genital damage to the female (Crudgington and Siva-Jothy, 2000), or force the female to mate at a suboptimal rate (Arnqvist and Nilsson, 2000; Arnqvist and Rowe, 2002a). There is good evidence demonstrating that females may pay substantial fitness costs of mating, such as reduced longevity and/or offspring production (Arnqvist and Rowe, 2005; Fiumera et al., 2006; Gavrilets et al., 2001). Males may also try to exploit the females' perception system during mate choice and parental investment. Exploitation of females may be most successful through exaggerated sexually selected traits in males, such as ornaments or song. Females are expected to counter-adapt through more selective mate choice (Arnqvist and Rowe, 2005; Chapman et al., 2003; Gavrilets et al., 2001) leading to female resistance to mating and the evolution of exaggerated male display to overcome this resistance (sexually antagonistic coevolution, Chapman et al., 2003; Holland and Rice, 1998). In that light, the evolutionary driving force of preference is resistance to male-imposed costs, rather than gaining benefits from mating with preferred males, as described under classic sexual selection (Chapman et al., 2003; Gavrilets et al., 2001; Holland and Rice, 1998). Mediated

by this dynamic process of manipulation and resistance, sexual conflict may have a pronounced influence on the evolution of both morphological and behavioural traits.

The prediction from sexual conflict theory that such a process of manipulation and resistance would be more intense resulting in greater sexual dimorphism when parents exhibit conflict over reproduction, but not when parents cooperate, is supported by our results: male EPTs may try to manipulate their partner *via* elaborate plumage and song. This, in conjunction with resistance by the female, may have led to the exaggeration of these traits in EPT, but not in CPT.

Our prediction that the intensity of sexual selection increases with more polygamous breeding systems (Andersson, 1994; Björklund, 1990; Székely et al., 2006) was supported by our results with regards to the differences in plumage dimorphism and song complexity, but is inconsistent with our result that females pay a cost of mating to an attractive male relative to mating with a less attractive male. We thus suggest that the process of manipulation and resistance should be considered as a valid alternative explanation to the traditional view of sexual selection in explaining the evolution of sexual dimorphism and song complexity.

Another alternative explanation for trait elaboration in EPT may be the higher population density and the apparently more male-biased population sex ratio, and thus probably a more biased operational sex ratio than in CPT (OSR, Emlen and Oring, 1977); see Methods for number of males and females; females care more than males, see Introduction). Several authors have suggested that higher population density and a biased OSR promote the opportunity for and higher levels of sexual selection by increasing competition for mates (e.g. Emlen and Oring, 1977; Kvarnemo and Ahnesjö, 1996; Owens, 2002), but the generality of this mechanism has been challenged recently (Head et al., 2008; Kokko and Rankin, 2006).

Finally, we found that the extent of cooperation in the breeding system was reflected in parental behaviour, quantified as the time spent on nest attendance. As expected, we found that EPT parents not only spend less time overall on nest attendance than CPT parents, but they also visited the nest less synchronously. The latter was also true when we compared the estimated absolute time the parents spent jointly at the nest, confirming that the difference in day length between our two study sites does not alter our results. These results suggest that EPT parents may try to avoid each other at the nest, in order to force the partner to do the work, as expected from sexual conflict theory. The energy saved as such may then be invested in a next reproductive bout in EPT, whereas in

CPT, where there are fewer opportunities for future reproduction, parents would benefit from more intense mutual cooperation at a given nest. The fact that the total time spent on nest attendance is lower in EPT than in CPT corroborates the idea that they actively try to avoid each other, rather than taking over each other's work as a cooperative effort. We note that CPTs did not spend more time together at the nest than expected by chance, so it seems that CPTs do not actively cooperate in nest attendance. We acknowledge that the relatively low power within CPT makes our latter statement not unambiguous, yet the sample size required to detect a statistically significant difference is unrealistically large, whereas if the effect size of the behaviour in CPT would have been of a similar extent as in EPT, we would have been able to detect this. A potential alternative explanation may be the differences in mate guarding between the species investigated. However, although we do not know the levels of extra-pair fertilizations in the two species, one would expect mate guarding to be more intense in EPT as levels of promiscuity are likely higher than in CPT given the EPT's polygamous breeding system, and because EPTs live in a much denser population (Kempenaers et al., 1995; Møller and Ninni, 1998). Our behavioural observations suggest the contrary.

In sum, we show that sexual conflict may be a major drive behind the evolution of various morphological and behavioural traits in penduline tits. Although we found support for various predictions of sexual conflict theory, we acknowledge that the generality of our conclusions is constrained by the limited sample of species included in this study. We have also not investigated the importance of ecological differences in mediating divergence in breeding systems. The reed marshes in Hungary appear to be rich in food, whereas the scrubland habitat in South Africa seems poor (R. E. van Dijk, Á. Pogány, T. Székely, personal observation). These ecological differences may have confounded the behaviour studied here, although one would expect that if food resources are plentiful (as in EPT), parents would spend less time away from the nest than when food is scarce (CPT). The pattern we found in nest attendance suggests the contrary: parents in food rich habitats (EPT) spend more time away from the nest.

An environment with high food availability, where one parent may be sufficient to raise the offspring, provides an opportunity for one of the parents to desert (Beissinger and Snyder, 1987; Olson et al., 2008; Székely et al., 1996; van Dijk and Székely, 2008). The resulting polygamous breeding system with overt sexual conflict may then act on the evolution of sexually selected traits as described in this paper. In order to establish the generality of our results, future studies may focus on phylogenetic comparative analyses,

testing competing hypotheses whilst controlling for multiple confounding variables. This approach can also be used to investigate various ecological (e.g. climate and predation rate) and genetic variables (e.g. kinship and genetic compatibility) that may drive the levels of conflict or cooperation in a species' breeding system.

IX CONCLUSIONS AND FUTURE DIRECTIONS

1. The evolution of sexual signals *via* sexual selection and sexually antagonistic coevolution in Eurasian penduline tits

In the aviary and in field experiments, I investigated three male traits of Eurasian penduline tits, and found that two of them are likely to be involved in sexual selection.

Mask size

Corroborating the conclusions of a field study (Kingma et al., 2008) females preferred dummy males with large masks (*Chapter III*). Eurasian penduline tits are polygamous, therefore the benefits for males with large masks multiply; they not only find mates faster but have more mates in a breeding season than those males with small masks (Kingma et al., 2008). From the females' perspective, however, I found a cost related to this preference in terms of parental care, because females mated to males with large masks were more frequently deserted than those mated to males with small masks (*Chapter VIII*). Male mask size therefore is associated with increased male reproductive success, although it probably decreases the reproductive success of females. The antagonistic effects of male mask size on males and females are consistent with predictions of sexual conflict theory. These results therefore suggest that male mask size is a trait that evolves – at least partly – by sexually antagonistic coevolution, i.e. by the process of manipulation by males and resistance by females (Arnqvist and Rowe, 2005; Chapman et al., 2003; Gavrilets et al., 2001).

No study has yet investigated the possible direct or indirect benefits that females may gain by choosing males with large masks, although it is unlikely that the high direct costs of mating with a manipulative male is counter-balanced by other direct or indirect benefits for females (e.g. good-manipulating sons and thus more grand-offspring, Cameron et al., 2003; Chapman, 2006; Chapman et al., 2003; Cordero and Eberhard, 2003). Future studies are needed to investigate the relative costs and benefits linked to male manipulation and female resistance, and Eurasian penduline tits appear to be a suitable study species to investigate the above assumptions.

In *Chapter IV* I also tested whether mask size in Eurasian penduline tits function in, and thus evolve by, male-male contests. Consistent with a field experiment (Kingma et al.,

2008) I found no evidence that this is the case. Furthermore, resident males with large masks did not react more strongly to intruders than those with small masks (*Chapter V*). Therefore, it appears that male mask size has no dual function (Berglund et al., 1996), and is involved only in intersexual communication in Eurasian penduline tits.

Nest size

The mate choice test (*Chapter III*) showed no preference for nest size. This male trait is associated with direct benefits for females, since larger nests have better insulation and consequently puts less energetic demand on the incubating parent (Szentirmai et al., 2005b). Although my finding is in contrast with earlier field observations by Hoi et al. (1994) and Schleicher et al. (1996), these studies compared the size of the nests of males that acquired mates with those of males that were not successful in mating. Mate choice typically occurs in an intermediate stage of nest-building in Eurasian penduline tits, and the couple finishes the nest together. Therefore, the above studies were probably confounded by female (and male) nest-building after mating (Persson and Öhrström, 1996; but see Hoi et al., 1996), whereas our test in controlled aviary conditions was not compromised by male or female behaviour. An interesting alternative explanation to the discrepancy is that female preference for nest size may be context-dependent (Cotton et al., 2006), and only in relatively cold ambient weather females may pay attention to this male trait whereas this preference was probably kept hidden in the mild conditions of our aviary setup. Although many research focused on explaining the variance of the male traits involved in sexual selection, much less is known about the variance in female preference. Since female choice is a core feature of male-female coevolution, it would be fruitful to pay more attention to this part of the process in the future.

Acoustic signals

Chapter V revealed that song in Eurasian penduline tits is involved in male-male communication. On the one hand, this finding is in line with studies investigating the unique mating and breeding system of Eurasian penduline tits, as they have long used the behavioural response that male penduline tits give to song playback and a male dummy close to their nest in order to catch and ring penduline tits (e.g. Bleeker et al., 2005; Kingma et al., 2008; Szentirmai et al., 2007; van Dijk et al., 2007). Here I investigated two possible ways that male song may communicate. Contrary to my assumption, neither intruder repertoire size nor song type elicited different defensive behaviour from resident

males. On the other hand, our study showed consistent behaviour by resident males in intruder situations, and the intensity and aggression of behavioural responses towards intruders were explained by the resident male's own repertoire size. This suggests that repertoire size is an honest signal in Eurasian penduline tits, by which resident males signal their competitive ability to other males in the population.

Although females seemed not to be interested in extra-pair copulations based on intruder male songs close to their nest, unmated and mated males sing vigorously around their nest probably to attract females and strengthen pair bond. It is very likely therefore, that song is also involved in intrasexual communication in Eurasian penduline tits. Because of the increasing complexity of the aviary mate choice test in *Chapter III*, I restricted the investigated male traits to mask size and nest size, and controlled for the effects of male song. To follow up the above line of studies, future research may test the multiple cues hypothesis in Eurasian penduline tits (Candolin, 2003), and investigate the role of male song besides mask size. It would be especially interesting to study whether male song – similarly to mask size – is also a manipulative trait, or mixed evolutionary processes (sexually antagonistic coevolution in male mask size, whereas directional sexual selection in male song) within the same species are responsible for shaping the evolution of multiple cues involved in male-female coevolution.

2. Sex differences and patterns of care in Eurasian penduline tits

Are females better parents than males?

In studies of different populations of the Eurasian penduline tit, a consistent female-bias in uniparental care was observed. Interpreting this pattern in light of the intense sexual conflict over parental care in this species suggest that males are better in achieving the more favourable outcome of the conflict over care than females (Szentirmai et al., 2007). However, a possible alternative explanation is that females are better parents and are selected to provide care (Erckmann, 1983). In *Chapter VI* I investigated this proposition, however, my results did not support this hypothesis, as male and female parents seem to be similar in their parental abilities. I did not find a significant difference between males and females in incubation or provisioning, and neither was there a difference in chick survival and development in male-cared and female-cared nests. Although based on reasonable

sample sizes, my study used a correlative approach and field data to compare male and female parental qualities. Since male-cared and female-cared nests naturally differ in clutch size (*Chapter VI*), this hindered the adequate comparison of clutch survival in large clutches. A more robust approach of experimental manipulations (e.g. the artificial enlargement of male-cared clutches) is needed to support the conclusion of similar male and female care.

It seems reasonable to assume that male Eurasian penduline tits more frequently achieve the favourable outcome of post-zygotic sexual conflict, as indicated by my above findings. What, then, are the reasons for this pattern? And what prevents females evolving to counter-balance the costly male behaviour? A possible drive behind this bias may be the sexually different costs and benefits of offspring desertion. In many species, the reproductive success of males increase more strongly with the number of mates than does that of females (Andersson, 1994; Bateman, 1948). In Eurasian penduline tits, female-cared clutches are approximately 1.5 times larger and consequently fledge more young than those of male-cared nests (*Chapter VI*, van Dijk et al., 2007). This is because a female keeps laying eggs after her mate has deserted, whereas if the female deserts, the male is stuck with a limited number of eggs. This suggests that in Eurasian penduline tits, selection to leave the care of the current clutch to the former mate in order to find a new mate is stronger for males than for females; in other words, the Bateman gradient is higher for males than for females (Andersson and Iwasa, 1996; Bateman, 1948).

How does intra-population variance in parental care build up in Eurasian penduline tits?

In species with a variable breeding system, such as the Eurasian penduline tit, either individually consistent or individually variable parental decisions may be responsible for variance at the population level. Investigating the flexibility of parental decisions on an individual level may reveal how much environmental influences may contribute to these decisions. In *Chapter VII*, I used a randomization approach to address this question, and found sexually different strategies in desertion behaviour. Female care seems to be less flexible, and it appears that there are two types of females: some of them consistently care for their nests, whereas others consistently desert them. Male behaviour, on the other hand, is more unique, but at the same time it seems to be influenced more by environmental changes and is thus more flexible in response to these changes. Males start the breeding season by deserting their nests, and later in the season some of them start to care for their offspring.

Which are the evolutionary processes maintaining the differing male and female strategies and which physiological mechanisms are responsible for the observed patterns? Theoretical modelling of this unique breeding system may help us to understand the underlying evolutionary processes. Since reproductive success of both males and females depends on the parental decision of their mate and of other individuals in the population (Szentirmai et al., 2007), this requires a game-theoretical approach (Maynard Smith, 1982). To address questions concerning the mechanisms, a promising candidate is to look at gene-environment-hormone interactions. The dynamically developing field of neuroendocrinology has recently gained a lot of interest by evolutionary biologists, because its new techniques allow us to pin down the elements of complex behaviours such as parental care (Adkins-Regan, 2005).

3. Evolutionary consequences of conflict and cooperation in penduline tits

Sexual conflict theory predicts antagonistic coevolution of males and females, which in turn affects the morphology and behaviour of the species, influences breeding system evolution and may even contribute to speciation (Arnqvist and Rowe, 2005; Chapman et al., 2003). The two closely-related penduline tit species investigated in *Chapter VIII* provide an excellent opportunity to study the evolutionary footprints of sexual conflict, since the Eurasian penduline tit represents a species with strong sexual conflict, whereas the Cape penduline tit exhibits intense cooperation. Comparing these two species I found differences in morphology and behaviour which are consistent with predictions of sexual conflict theory. Eurasian penduline tits exhibited sexual dimorphism in plumage, and more variable song than Cape penduline tits. Cape penduline tits, on the other hand, were more cooperative in their nest attendance.

For the effects on Figure I.1 of Introduction (page 11), I found support for all the expected outcomes of bias towards either sexual conflict or cooperation in penduline tits. The intensity of sexual selection and sexual dimorphism, parental care and breeding system in these penduline tit species were all in line with the predictions of sexual conflict theory. The next question would be to track down the selective forces responsible for evolution of a species towards either more conflict or more cooperation. Although the reported differences between Eurasian and Cape penduline tits are striking, the study

involved only two species and so the validity of these results beyond these two species needs to be confirmed. Future studies may expand the comparison with more penduline tit species (*Remizinae*) inhabiting different habitats, and comparative phylogenetic approach may reveal whether sexual conflict indeed is fuel for sexual selection and breeding system evolution, as suggested by the above findings.

REFERENCES

Adkins-Regan E, 1999. Testosterone increases singing and aggression but not male-typical sexual partner preference in early estrogen treated female zebra finches. *Hormones and Behavior* 35:63-70.

Adkins-Regan E, 2005. Hormones and animal social behavior. Princeton: Princeton University Press.

Alström P, Ericson PGP, Olsson U, Sundberg P, 2006. Phylogeny and classification of the avian superfamily *Sylvioidea*. *Molecular Phylogenetics and Evolution* 38:381-397.

Andersson M, 1994. Sexual selection. Princeton: Princeton University Press.

Andersson M, Iwasa Y, 1996. Sexual selection. *Trends in Ecology & Evolution* 11:53-38.

Andersson M, Simmons LW, 2006. Sexual selection and mate choice. *Trends in Ecology & Evolution* 21:296-302.

Arnold SJ, Duvall D, 1994. Animal mating systems - A synthesis based on selection theory. *American Naturalist* 143:317-348.

Arnqvist G, Edvardsson M, Friberg U, Nilsson T, 2000. Sexual conflict promotes speciation in insects. *Proceedings of the National Academy of Sciences of the United States of America* 97:10460-10464.

Arnqvist G, Kirkpatrick M, 2005. The evolution of infidelity in socially monogamous passerines: The strength of direct and indirect selection on extrapair copulation behavior in females. *American Naturalist* 165:S26-S37.

Arnqvist G, Nilsson T, 2000. The evolution of polyandry: Multiple mating and female fitness in insects. *Animal Behaviour* 60:145-164.

Arnqvist G, Rowe L, 2002a. Antagonistic coevolution between the sexes in a group of insects. *Nature* 415:787-789.

Arnqvist G, Rowe L, 2002b. Correlated evolution of male and female morphologies in water striders. *Evolution* 56:936-947.

Arnqvist G, Rowe L, 2005. Sexual conflict. Princeton: Princeton University Press.

Badyaev AV, Duckworth RA, 2005. Evolution of plasticity in hormonally-integrated parental tactics: An example with the house finch. In: Functional avian endocrinology (Dawson A, Sharp PJ, eds). New Delhi, India: Narosa Publishing House.

Ballentine B, Hyman J, Nowicki S, 2004. Vocal performance influences female response to male bird song: an experimental test. *Behavioral Ecology* 15:163-168.

Balsby TJS, Dabelsteen T, 2001. The meaning of song repertoire size and song length to male whitethroats *Sylvia communis*. *Behavioural Processes* 56:75-84.

Balshine-Earn S, 1995. The costs of parental care in Galilee St Peter's fish, *Sarotherodon galilaeus*. *Animal Behaviour* 50:1-7.

Balshine-Earn S, Kempenaers B, Székely T, 2002. Conflict and cooperation in parental care - introduction. *Philosophical Transactions of the Royal Society of London B* 357:237-240.

Bart J, Tornes A, 1989. Importance of monogamous male birds in determining reproductive success - Evidence for house wrens and a review of male-removal studies. *Behavioral Ecology and Sociobiology* 24:109-116.

Barta Z, Houston AI, McNamara JM, Székely T, 2002. Sexual conflict about parental care: the role of reserves. *American Naturalist* 159:687-705.

Bateman AJ, 1948. Intra-sexual selection in Drosophila. *Heredity* 2:349-368.

Beebee MD, 2004. The functions of multiple singing modes: Experimental tests in yellow warblers, *Dendroica petechia*. *Animal Behaviour* 67:1089-1097.

Beecher MD, Campbell SE, Burt JM, Hill CE, Nordby JC, 2000. Song-type matching between neighbouring song sparrows. *Animal Behaviour* 59:21-27.

Beissinger SR, Snyder NFR, 1987. Mate desertion in the snail kite. *Animal Behaviour* 35:477-487.

Bennett ATD, Cuthill IC, Norris KJ, 1994. Sexual selection and the mismeasure of color. *American Naturalist* 144:848-860.

Bennett ATD, Thery M, 2007. Avian color vision and coloration: Multidisciplinary evolutionary biology. *American Naturalist* 169:S1-S6.

Berglund A, Bisazza A, Pilastro A, 1996. Armaments and ornaments: An evolutionary explanation of traits of dual utility. *Biological Journal of the Linnean Society* 58:385-399.

Berglund A, Rosenqvist G, 2003. Sex role reversal in pipefish. *Advances in the Study of Behavior* 32:131-167.

Björklund M, 1990. A phylogenetic interpretation of sexual dimorphism in body size and ornament in relation to mating system in birds. *Journal of Evolutionary Biology* 3:171-183.

Bleeker M, Kingma SA, Szentirmai I, Székely T, Komdeur J, 2005. Body condition and clutch desertion in penduline tit *Remiz pendulinus*. *Behaviour* 142:1465-1478.

Blouw DM, 1996. Evolution of offspring desertion in a stickleback fish. *Ecoscience* 3:18-24.

Bókony V, Liker A, Székely T, Kis J, 2003. Melanin-based plumage coloration and flight displays in plovers and allies. *Proceedings of the Royal Society of London B* 270:2491-2497.

Box GEP, Hunter JS, Hunter WG, 2005. Statistics for experimenters: Design, innovation, and discovery, 2nd ed. Hoboken, N.J.: Wiley-Interscience.

Brooks R, Endler JA, 2001. Female guppies agree to differ: Phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution* 55:1644-1655.

Brosnan SF, de Waal FBM, 2003. Monkeys reject unequal pay. *Nature* 425:297-299.

Burley NT, 1988. The differential-allocation hypothesis - An experimental test. *American Naturalist* 132:611-628.

Calkins JD, Burley NT, 2003. Mate choice for multiple ornaments in the California quail, *Callipepla californica*. *Animal Behaviour* 65:69-81.

Cameron E, Day T, Rowe L, 2003. Sexual conflict and indirect benefits. *Journal of Evolutionary Biology* 16:1055-1060.

Candolin U, 2003. The use of multiple cues in mate choice. *Biological Reviews of the Cambridge Philosophical Society* 78:575-595.

Catchpole CK, 1989. Responses of male sedge warblers to playback of different repertoire sizes. *Animal Behaviour* 37:1046-1047.

Catchpole CK, 1996. Song and female choice: Good genes and big brains? *Trends in Ecology & Evolution* 11:358-360.

Catchpole CK, Dittami J, Leisler B, 1984. Differential responses to male song repertoires in female songbirds implanted with estradiol. *Nature* 312:563-564.

Catchpole CK, Slater PJB, 2008. Bird song: Biological themes and variations. Cambridge: Cambridge University Press.

Chapman T, 2006. Evolutionary conflicts of interest between males and females. *Current Biology* 16:R744-R754.

Chapman T, Arnqvist G, Bangham J, Rowe L, 2003. Sexual conflict. *Trends in Ecology & Evolution* 18:41-47.

Charmantier A, Keyser AJ, Promislow DEL, 2007. First evidence for heritable variation in cooperative breeding behaviour. *Proceedings of the Royal Society of London B* 274:1757-1761.

Clutton-Brock TH, 1991. The evolution of parental care. Princeton: Princeton University Press.

Clutton-Brock TH, 2007. Sexual selection in males and females. *Science* 318:1882-1885.

Cohen J, 1988. Statistical power analysis for the behavioral sciences, 2nd ed. Hillsdale: Lawrence Erlbaum Associates.

Colman AM, 2006. The puzzle of cooperation. *Nature* 440:744-745.

Conway CJ, Martin TE, 2000. Effects of ambient temperature on avian incubation behavior. *Behavioral Ecology* 11:178-188.

Cordero C, Eberhard G, 2003. Female choice of sexually antagonistic male adaptations: a critical review of some current research. *Journal of Evolutionary Biology* 16:1-6.

Cotton S, Small J, Pomiankowski A, 2006. Sexual selection and condition-dependent mate preferences. *Current Biology* 16:R755-R765.

Cramp S, Perrins CM, Brooks DJ, 1993. Handbook of the birds of Europe, the Middle East and North Africa - Birds of the Western Palearctic. Oxford: Oxford University Press.

Crudgington HS, Siva-Jothy MT, 2000. Genital damage, kicking and early death - The battle of the sexes takes a sinister turn in the bean weevil. *Nature* 407:855-856.

Dale J, 2006. Intraspecific variation in bird colors. In: Bird coloration (Hill GE, McGraw KJ, eds). Cambridge: Harvard University Press.

Dale S, Slagsvold T, 1996. Mate choice on multiple cues, decision rules and sampling strategies in female pied flycatchers. *Behaviour* 133:903-944.

Darwin CR, 1859. On the origin of species by means of natural selection. London: Murray.

Darwin CR, 1871. The descent of man and selection in relation to sex. London: Murray.

Davies NB, 1989. Sexual conflict and the polygamy threshold. *Animal Behaviour* 38:226-234.

Dawkins R, 1976. The selfish gene. Oxford: Oxford University Press.

Dawkins R, Carlisle TR, 1976. Parental investment, mate desertion and a fallacy. *Nature* 262:131-133.

Dean WRJ, 2005. Cape penduline tit *Anthoscopus minutus*. In: Roberts birds of Southern Africa (Hockey PAR, Dean WRJ, Ryan PG, eds). Cape Town: John Voelker Bird Book Fund.

Dingemanse NJ, Both C, Drent PJ, van Oers K, van Noordwijk AJ, 2002. Repeatability and heritability of exploratory behaviour in great tits from the wild. *Animal Behaviour* 64:929-938.

Dobbs RC, Styrsky JD, Thompson CF, 2006. Clutch size and the costs of incubation in the house wren. *Behavioral Ecology* 17:849-856.

Eckert CG, Weatherhead PJ, 1987. Male characteristics, parental quality and the study of mate choice in the red-winged blackbird (*Agelaius phoenicus*). *Behavioral Ecology and Sociobiology* 20:35-42.

Efstratiadis A, 1994. Parental imprinting of autosomal mammalian genes. *Current Opinion in Genetics & Development* 4:265-280.

Emlen ST, Oring LW, 1977. Ecology, sexual selection, and evolution of mating systems. *Science* 197:215-223.

Endler JA, 1990. On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society* 41:315–352.

Erckmann WJ, 1983. The evolution of polyandry in shorebirds: an evaluation of hypotheses. In: Social behavior of female vertebrates (Wasser SK, ed). New York: Academic Press.

Fiumera AC, Dumont BL, Clark AG, 2006. Natural variation in male-induced 'cost-of-mating' and allele-specific association with male reproductive genes in *Drosophila melanogaster*. *Philosophical Transactions of the Royal Society of London B* 361:355-361.

Foerster K, Kempenaers B, 2005. Effects of testosterone on male-male competition and male-female interactions in blue tits. *Behavioral Ecology and Sociobiology* 57:215-223.

Forsgren E, Amundsen T, Borg AA, Bjelvenmark J, 2004. Unusually dynamic sex roles in a fish. *Nature* 429:551-554.

Franz D, 1991. Paarungssystem und fortpflanzunsstrategie der beutelmeise (*Remiz p. pendulinus*). *Journal of Ornithology* 132:241-266.

Franz D, Theiss N, 1983. Brutbiologie und bestandsentwicklung einer farbberingten population der Beutelmeise (*Remiz pendulinus*). *Verhandlungen der Ornithologischen Gesselschaft in Bayern* 23:393-442.

Freeman-Gallant CR, Rothstein MD, 1999. Apparent heritability of parental care in Savannah sparrows. *Auk* 116:1132-1136.

Frere E, Gandini P, Boersma D, 1998. The breeding ecology of Magellanic penguins at Cabo Virgenes, Argentina: what factors determine reproductive success? *Colonial Waterbirds* 21:205-210.

Fuller RC, Houle D, Travis J, 2005. Sensory bias as an explanation for the evolution of mate preferences. *American Naturalist* 166:437-446.

Gavrilets S, Arnqvist G, Friberg U, 2001. The evolution of female mate choice by sexual conflict. *Proceedings of the Royal Society of London B* 268:531-539.

Gill FB, Slikas B, Sheldon FH, 2005. Phylogeny of titmice (*Paridae*): II. Species relationships based on sequences of the mitochondrial cytochrome-b gene. *Auk* 122:121-143.

Gonzalez-Voyer A, Fitzpatrick JL, Kolm N, 2008. Sexual selection determines parental care patterns in cichlid fishes. *Evolution* 62:2015-2026.

Griffith SC, Parker TH, Olson VA, 2006. Melanin-versus carotenoid-based sexual signals: is the difference really so black and red? *Animal Behaviour* 71:749-763.

Griffiths R, Double MC, Orr K, Dawson RJG, 1998. A DNA test to sex most birds. *Molecular Ecology* 7:1071-1075.

Griggio M, Serra L, Licheri D, Monti A, Pilastro A, 2007. Armaments and ornaments in the rock sparrow: a possible dual utility of a carotenoid-based feather signal. *Behavioral Ecology and Sociobiology* 61:423-433.

Grubbauer P, 1995. The importance of the nest in reproductive success and mate choice for a uniparental incubator. A study in the penduline tit *Remiz pendulinus*. M. D. Thesis, Vienna: University of Vienna.

Grubbauer P, Hoi H, 1996. Female penduline tits (*Remiz pendulinus*) choosing high quality nests benefit by decreased incubation effort and increased hatching success. *Ecoscience* 3:274-279.

Grüter C, Taborsky B, 2005. Sex ratio and the sexual conflict about brood care in a biparental mouthbrooder. *Behavioral Ecology and Sociobiology* 58:44-52.

Hadfield JD, Burgess MD, Lord A, Phillimore AB, Clegg SM, Owens IPF, 2006. Direct *versus* indirect sexual selection: genetic basis of colour, size and recruitment in a wild bird. *Proceedings of the Royal Society of London B* 273:1347-1353.

Haftorn S, Reinertsen RE, 1985. The effect of temperature and clutch size on the energetic cost of incubation in a free-living blue tit (*Parus caeruleus*). *Auk* 102:470-478.

Haig D, 1997. Parental antagonism, relatedness asymmetries, and genomic imprinting. *Proceedings of the Royal Society of London B* 264:1657-1662.

Hamilton PS, Sullivan BK, 2005. Female mate attraction in ornate tree lizards, *Urosaurus omatus*: a multivariate analysis. *Animal Behaviour* 69:219-224.

Haraszthy L, 2000. Magyarország madarai, 2nd ed. Budapest: Mezőgazda kiadó.

Harrap S, Quinn D, 1996. Tits, nuthatches and treecreepers. London: Christopher Helm.

Head ML, Lindholm AK, Brooks R, 2008. Operational sex ratio and density do not affect directional selection on male sexual ornaments and behavior. *Evolution* 62:135-144.

Heinsohn R, Packer C, 1995. Complex cooperative strategies in group-territorial African lions. *Science* 269:1260-1262.

Hill GE, Brawner WE, 1998. Melanin-based plumage coloration in the house finch is unaffected by coccidial infection. *Proceedings of the Royal Society of London B* 265:1105-1109.

Hoi H, Schleicher B, Valera F, 1994. Female mate choice and nest desertion in penduline tits, *Remiz pendulinus* - The importance of nest quality. *Animal Behaviour* 48:743-746.

Hoi H, Schleicher B, Valera F, 1996. Nest size variation and its importance for mate choice in penduline tits, *Remiz pendulinus*. *Animal Behaviour* 51:464-466.

Holland B, Rice WR, 1998. Perspective: Chase-away sexual selection: Antagonistic seduction *versus* resistance. *Evolution* 52:1-7.

Hosken D, Snook R, 2005. How important is sexual conflict? *American Naturalist* 165:S1-S4.

Hosken DJ, Stockley P, 2004. Sexual selection and genital evolution. *Trends in Ecology & Evolution* 19:87-93.

Houston AI, Davies NB, 1985. The evolution of cooperation and life history in the dunnock *Prunella modularis*. In: Behavioural Ecology (Sibley RM, Smith RH, eds). Oxford: Blackwell Scientific Publications.

Houston AI, Székely T, McNamara JM, 2005. Conflict between parents over care. *Trends in Ecology & Evolution* 20:33-38.

Innocent TM, West SA, 2006. Social evolution: Cooperation by conflict. *Current Biology* 16:R365-R367.

Jawor JM, Breitwitsch R, 2003. Melanin ornaments, honesty and sexual selection. *Auk* 120:249-265.

Jennions MD, Petrie M, 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews of the Cambridge Philosophical Society* 72:283-327.

Jennions MD, Petrie M, 2000. Why do females mate multiply? A review of the genetic benefits. *Biological Reviews of the Cambridge Philosophical Society* 75:21-64.

Jensen K, Call J, Tomasello M, 2007. Chimpanzees are rational maximizers in an ultimatum game. *Science* 318:107-109.

Jones IL, Hunter FM, 1999. Experimental evidence for mutual inter- and intrasexual selection favouring a crested auklet ornament. *Animal Behaviour* 57:521-528.

Kempenaers B, Verheyen GR, Dhondt AA, 1995. Mate guarding and copulation behavior in monogamous and polygynous blue tits - Do males follow a best-of-a-bad-job strategy? *Behavioral Ecology and Sociobiology* 36:33-42.

Ketterson ED, Nolan V, Wolf L, Ziegenfus C, 1992. Testosterone and avian life histories - Effects of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (*Junco hyemalis*). *American Naturalist* 140:980-999.

Kingma SA, Szentirmai I, Székely T, Bókony V, Bleeker M, Liker A, Komdeur J, 2008. Sexual selection and the function of a melanin-based plumage ornament in polygamous penduline tits *Remiz pendulinus*. *Behavioral Ecology and Sociobiology* 62:1277-1288.

Kirkpatrick M, Ryan MJ, 1991. The evolution of mating preferences and the paradox of the lek. *Nature* 350:33-38.

Kodric-Brown A, Nicoletto PF, 2001. Female choice in the guppy (*Poecilia reticulata*): the interaction between male color and display. *Behavioral Ecology and Sociobiology* 50:346-351.

Kokko H, 2001. Fisherian and "good genes" benefits of mate choice: how (not) to distinguish between them. *Ecology Letters* 4:322-326.

Kokko H, Brooks R, McNamara JM, Houston AI, 2002. The sexual selection continuum. *Proceedings of the Royal Society of London B* 269:1331-1340.

Kokko H, Jennions M, 2003. It takes two to tango. *Trends in Ecology & Evolution* 18:103-104.

Kokko H, Jennions MD, 2008. Parental investment, sexual selection and sex ratios. *Journal of Evolutionary Biology* 21:919-948.

Kokko H, Rankin DJ, 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philosophical Transactions of the Royal Society of London B* 361:319-334.

Komdeur J, Heg D, 2005. Cooperation and conflict over investment strategies in animals. *Behaviour* 142:1433-1447.

Kölliker M, Richner H, 2001. Parent-offspring conflict and the genetics of offspring solicitation and parental response. *Animal Behaviour* 62:395-407.

Krebs JR, Ashcroft R, Vanorsdol K, 1981. Song matching in the great tit *Parus major L*. *Animal Behaviour* 29:918-923.

Krebs JR, Ashcroft R, Webber M, 1978. Song repertoires and territory defence in great tit. *Nature* 271:539-542.

Kroodsma DE, 1982. Song repertoires: problems in their definition and use. In: Acoustic communication in birds (Kroodsma DE, Miller EH, eds). New York: Academic Press.

Kroodsma DE, 1989. Suggested experimental designs for song playbacks. *Animal Behaviour* 37:600-609.

Kroodsma DE, Byers BE, 1991. The function(s) of bird song. *American Zoologist* 31:318-328.

Kümmerli R, Colliard C, Fiechter N, Petitpierre B, Russier F, Keller L, 2007. Human cooperation in social dilemmas: Comparing the snowdrift game with the prisoner's dilemma. *Proceedings of the Royal Society of London B* 274:2965-2970.

Kvarnemo C, Ahnesjö I, 1996. The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution* 11:404-408.

Lambrechts MM, 1992. Male quality and playback in the great tit. In: Playback and studies of animal communication (McGregor PK, ed). New York: Plenum Press.

Lambrechts MM, Perret P, 2000. A long photoperiod overrides non-photoperiodic factors in blue tits' timing of reproduction. *Proceedings of the Royal Society of London B* 267:585-588.

Lazarus J, 1990. The logic of mate desertion. *Animal Behaviour* 39:672-684.

Legge S, 1996. Cooperative lions escape the prisoner's dilemma. *Trends in Ecology & Evolution* 11:2-3.

Lehmann EL, 1975. Nonparametrics. Statistical methods based on ranks. San Francisco: Holden-Day.

Lendvai AZ, Kis J, Székely T, Cuthill IC, 2004. An investigation of mate choice based on manipulation of multiple ornaments in Kentish plovers. *Animal Behaviour* 67:703-709.

Lessells CM, 1999. Sexual conflict in animals. In: Levels of selection in evolution (Keller L, ed). Princeton: Princeton University Press.

Lessells CM, 2006. The evolutionary outcome of sexual conflict. *Philosophical Transactions of the Royal Society of London B* 361:301-317.

Lessells CM, Boag PT, 1987. Unrepeatable repeatabilities - A common mistake. *Auk* 104:116-121.

Lock JE, Smiseth PT, Moore AJ, 2004. Selection, inheritance, and the evolution of parent-offspring interactions. *American Naturalist* 164:13-24.

MacColl ADC, Hatchwell BJ, 2003a. Heritability of parental effort in a passerine bird. *Evolution* 57:2191-2195.

MacColl ADC, Hatchwell BJ, 2003b. Sharing of caring: nestling provisioning behaviour of long-tailed tit, *Aegithalos caudatus*, parents and helpers. *Animal Behaviour* 66:955-964.

Magrath MJL, Komdeur J, 2003. Is male care compromised by additional mating opportunity? *Trends in Ecology & Evolution* 18:424-430.

Manly BFJ, 2006. Randomization, bootstrap and Monte Carlo methods in biology, 3rd ed. London: Chapman & Hall.

Martin OY, Hosken DJ, 2003. The evolution of reproductive isolation through sexual conflict. *Nature* 423:979-982.

Maynard Smith J, 1977. Parental investment: A prospective analysis. *Animal Behaviour* 25:1-9.

Maynard Smith J, 1982. Evolution and the theory of games. Cambridge: Cambridge University Press.

McGraw KJ, 2006. Mechanics of melanin-based coloration. In: Bird coloration II. Function and evolution (Hill GE, McGraw KJ, eds). Cambridge, MA: Harvard University Press.

McGraw KJ, Mackillop EA, Dale J, Hauber ME, 2002. Different colors reveal different information: How nutritional stress affects the expression of melanin- and structurally based ornamental plumage. *Journal of Experimental Biology* 205:3747-3755.

McNamara JM, Houston AI, 2008. Optimal annual routines: behaviour in the context of physiology and ecology. *Philosophical Transactions of the Royal Society of London B* 363:301-319.

McNamara JM, Houston AI, Székely T, Webb JN, 2002. Do parents make independent decisions about desertion? *Animal Behaviour* 64:147-149.

McNamara JM, Székely T, Webb JN, Houston AI, 2000. A dynamic game-theoretic model of parental care. *Journal of Theoretical Biology* 205:605-623.

Møller AP, Ninni P, 1998. Sperm competition and sexual selection: a meta-analysis of paternity studies of birds. *Behavioral Ecology and Sociobiology* 43:345-358.

Møller AP, Pomiankowski A, 1993. Why have birds got multiple sexual ornaments? *Behavioral Ecology and Sociobiology* 32:167-176.

Monroe BL, Sibley CG, 1993. A world checklist of birds. New Heaven, USA: Yale University Press.

Nakagawa S, Gillespie DOS, Hatchwell BJ, Burke T, 2007. Predictable males and unpredictable females: sex difference in repeatability of parental care in a wild bird population. *Journal of Evolutionary Biology* 20:1674-1681.

Noë R, 2006. Cooperation experiments: coordination through communication *versus* acting apart together. *Animal Behaviour* 71:1-18.

Nowak MA, 2006. Five rules for the evolution of cooperation. *Science* 314:1560-1563.

Nowicki S, Searcy WA, Hughes M, 1998. The territory defense function of song in song sparrows: A test with the speaker occupation design. *Behaviour* 135:615-628.

Olson VA, Liker A, Freckleton RP, Székely T, 2008. Parental conflict in birds: comparative analyses of offspring development, ecology and mating opportunities. *Proceedings of the Royal Society of London B* 275:301-307.

Owens IPF, 2002. Male-only care and classical polyandry in birds: phylogeny, ecology and sex differences in remating opportunities. *Philosophical Transactions of the Royal Society of London B* 357:283-293.

Parker GA, 1979. Sexual selection and sexual conflict. In: Sexual selection and reproductive competition in insects (Blum MS, Blum NA, eds). New York: Academic Press.

Parker GA, 2006. Sexual conflict over mating and fertilization: An overview. *Philosophical Transactions of the Royal Society of London B* 361:235-259.

Parker GA, Baker RR, Smith VGF, 1972. The origin and evolution of gamete dimorphism and male-female phenomenon. *Journal of Theoretical Biology* 36:529-553.

Parker GA, Partridge L, 1998. Sexual conflict and speciation. *Philosophical Transactions of the Royal Society of London B* 353:261-274.

Parker TH, Stansberry BM, Becker CD, Gipson PS, 2003. Do melanin- or carotenoid-pigmented plumage ornaments signal condition and predict pairing success in the Kentucky warbler? *Condor* 105:663-671.

Pennisi E, 2005. How did cooperative behavior evolve? *Science* 309:93-93.

Pérez-Barbería FJ, Gordon IJ, Pagel M, 2002. The origins of sexual dimorphism in body size in ungulates. *Evolution* 56:1276-1285.

Persson O, Öhrström P, 1989. A new avian mating system: Ambisexual polygamy in the penduline tit (*Remiz pendulinus*). *Ornis Scandinavica* 20:105-111.

Persson O, Öhrström P, 1996. Female nest choice in the penduline tit: a comment on Hoi et al. (1994). *Animal Behaviour* 51:462-463.

Peters A, Astheimer LB, Boland CRJ, Cockburn A, 2000. Testosterone is involved in acquisition and maintenance of sexually selected male plumage in superb fairy-wrens, *Malurus cyaneus*. *Behavioral Ecology and Sociobiology* 47:438-445.

Peters A, Cockburn A, Cunningham R, 2002. Testosterone treatment suppresses paternal care in superb fairy-wrens, *Malurus cyaneus*, despite their concurrent investment in courtship. *Behavioral Ecology and Sociobiology* 51:538-547.

Pilastro A, Biddau L, Marin G, Mingozzi T, 2001. Female brood desertion increases with number of available mates in the rock sparrow. *Journal of Avian Biology* 32:68-72.

Poesel A, Dabelsteen T, 2006. Three vocalization types in the blue tit *Cyanistes caeruleus*: A test of the different signal-value hypothesis. *Behaviour* 143:1529-1545.

Pogány Á, Altbäcker V, Székely T, 2008a. Male signalling and dominance in the penduline tit *Remiz pendulinus*. *Acta Zoologica Academiae Scientiarum Hungaricae* 54:191-199.

Pogány Á, Székely T, 2007. Female choice in the penduline tit *Remiz pendulinus*: the effects of nest size and male mask size. *Behaviour* 144:411-427.

Pogány Á, Szentirmai I, Komdeur J, Székely T, 2008b. Sexual conflict and consistency of offspring desertion in Eurasian penduline tit *Remiz pendulinus*. *BMC Evolutionary Biology* 8:242.

Potti J, Moreno J, Merino S, 1999. Repeatability of parental effort in male and female pied flycatchers as measured with doubly labeled water. *Canadian Journal of Zoology* 77:174-179.

Prasad NG, Bedhomme S, Day T, Chippindale AK, 2007. An evolutionary cost of separate genders revealed by male-limited evolution. *American Naturalist* 169:29-37.

Price TAR, Hosken DJ, 2007. Evolution: Good males are bad females. *Current Biology* 17:R168-R170.

Queller DC, 1997. Why do females care more than males? *Proceedings of the Royal Society of London B* 264:1555-1557.

Qvarnström A, 2001. Context-dependent genetic benefits from mate choice. *Trends in Ecology & Evolution* 16:5-7.

Reichard UH, Boesch C, 2007. Monogamy: Mating strategies and partnership in birds, humans and other mammals. Cambridge: Cambridge University Press.

Reynolds JD, Goodwin NB, Freckleton RP, 2002. Evolutionary transitions in parental care and live bearing in vertebrates. *Philosophical Transactions of the Royal Society of London B* 357:269-281.

Rice WR, 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* 381:232-234.

Rice WR, Holland B, 1997. The enemies within: Intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behavioral Ecology and Sociobiology* 41:1-10.

Robertson IC, Roitberg BD, 1998. Duration of paternal care in pine engraver beetles: Why do larger males care less? *Behavioral Ecology and Sociobiology* 43:379-386.

Roff DA, 2002. Life history evolution. Massachusetts: Sinauer Associates.

Rowe L, Day T, 2006. Detecting sexual conflict and sexually antagonistic coevolution. *Philosophical Transactions of the Royal Society of London B* 361:277-285.

Rowe L, Ludwig D, Schluter D, 1994. Time, condition, and the seasonal decline of avian clutch size. *American Naturalist* 143:698-772.

Royle NJ, Hartley IR, Parker GA, 2002. Sexual conflict reduces offspring fitness in zebra finches. *Nature* 416:733-736.

Ryan MJ, 1998. Sexual selection, receiver biases, and the evolution of sex differences. *Science* 281:1999-2003.

Schleicher B, Hoi H, Valera F, 1996. Seasonal change in female mate choice criteria in penduline tits (*Remiz pendulinus*). *Ardeola* 43:19-29.

Schleicher B, Hoi H, Valera F, Hoi-Leitner M, 1997. The importance of different paternity guards in the polygynandrous penduline tit (*Remiz pendulinus*). *Behaviour* 134:941-959.

Schönfeld M, 1989. Beitraege zur biologie der beutelmeise, *Remiz pendulinus*. *Apus* 7:49-87.

Schroth M, Helbig A, 1985. Die beutelmeise (*Remiz pendulinus*), brutvogel in der untermainebene. *Vogel und Umwelt* 3:339-343.

Schwagmeyer PL, Mock DW, 2003. How consistently are good parents good parents? Repeatability of parental care in the house sparrow, *Passer domesticus*. *Ethology* 109:303-313.

Searcy WA, 1992. Song repertoire and mate choice in birds. *American Zoologist* 32:71-80.

Searcy WA, Andersson M, 1986. Sexual selection and the evolution of song. *Annual Review of Ecology and Systematics* 17:507-533.

Searcy WA, Nowicki S, 2000. Male–male competition and female choice in the evolution of vocal signalling. In: Animal signals: Signalling and signal design in animal communication (Espmark Y, Amundsen T, Rosenqvist G, eds). Trondheim: Tapir Academic Press.

Searcy WA, Nowicki S, Hogan C, 2000. Song type variants and aggressive context. *Behavioral Ecology and Sociobiology* 48:358-363.

Senar JC, 2006. Color displays as intrasexual signals of aggression and dominance. In: Bird coloration. II. Function and evolution (Hill GE, McGraw KJ, eds). Cambridge, MA: Harvard University Press.

Sheldon BC, 2000. Differential allocation: Tests, mechanisms and implications. *Trends in Ecology & Evolution* 15:397-402.

Siefferman L, Hill GE, 2003. Structural and melanin coloration indicate parental effort and reproductive success in male eastern bluebirds. *Behavioral Ecology* 14:855-861.

Simeone A, Araya B, Bernal M, Diebold EN, Grzybowski K, Michaels M, Teare JA, Wallace RS, Willis MJ, 2002. Oceanographic and climatic factors influencing breeding and colony attendance patterns of Humboldt penguins *Spheniscus humboldti* in Central Chile. *Marine Ecology Progress Series* 227:43-50.

Snyder BF, Gowaty PA, 2007. A reappraisal of Bateman's classic study of intrasexual selection. *Evolution* 61:2457-2468.

Sokal RR, Rohlf FJ, 1995. Biometry: The principles and practice of statistics in biological research, 3rd ed. New York: W. H. Freeman and Co.

Steinegger M, Taborsky B, 2007. Asymmetric sexual conflict over parental care in a biparental cichlid. *Behavioral Ecology and Sociobiology* 61:933-941.

Stephens DW, McLinn CM, Stevens JR, 2002. Discounting and reciprocity in an iterated prisoner's dilemma. *Science* 298:2216-2218.

Stoddard PK, Beecher MD, Willis MS, 1988. Response of territorial male song sparrows to song types and variation. *Behavioral Ecology and Sociobiology* 22:125-130.

Székely T, Thomas GH, Cuthill IC, 2006. Sexual conflict, ecology, and breeding systems in shorebirds. *Bioscience* 56:887-887.

Székely T, Webb JN, Houston AI, McNamara JM, 1996. An evolutionary approach to offspring desertion in birds. In: Current ornithology (Nolan V, Jr., Ketterson ED, eds). New York: Plenum Press.

Szentirmai I, 2005. Sexual conflict in penduline tit *Remiz pendulinus*. PhD thesis, Budapest: Eötvös University.

Szentirmai I, Komdeur J, Székely T, 2005a. What makes a nest-building male successful? Male behaviour and female care in penduline tits. *Behavioral Ecology* 16:994-1000.

Szentirmai I, Komdeur J, Székely T, 2007. Sexual conflict over care: antagonistic effect of clutch desertion on reproductive success of male and female penduline tits. *Journal of Evolutionary Biology* 20:1739-1744.

Szentirmai I, Székely T, Liker A, 2005b. The influence of nest size on heat loss of penduline tit eggs. *Acta Zoologica Academiae Scientiarum Hungaricae* 51:59-66.

Tallamy DW, 2001. Evolution of exclusive paternal care in arthopods. *Annual Review of Entomology* 46:139-165.

Tarof SA, Dunn PO, Whittingham LA, 2005. Dual functions of a melanin-based ornament in the common yellowthroat. *Proceedings of the Royal Society of London B* 272:1121-1127.

Tatarenkov A, Healey CIM, Grether GF, Avise JC, 2008. Pronounced reproductive skew in a natural population of green swordtails, *Xiphophorus helleri*. *Molecular Ecology* 17:4522-4534.

Taylor C, Nowak MA, 2007. Transforming the dilemma. *Evolution* 61:2281-2292.

Tomaszycki ML, Adkins-Regan E, 2005. Experimental alteration of male song quality and output affects female mate choice and pair bond formation in zebra finches. *Animal Behaviour* 70:785-794.

Trillo PA, Vehrencamp SL, 2005. Song types and their structural features are associated with specific contexts in the banded wren. *Animal Behaviour* 70:921-935.

Trivers RL, 1972. Parental investment and sexual selection. In: Sexual selection and the descent of man (Campbell B, ed). Chicago: Aldine Press.

Trivers RL, 1985. Social evolution. Menlo Park: Benjamin-Cummings.

Valera F, Hoi H, Schleicher B, 1997. Egg burial in penduline tits, *Remiz pendulinus*: Its role in mate desertion and female polyandry. *Behavioral Ecology* 8:20-27.

van Dijk RE, Pilon AE, Szentirmai I, Székely T, Komdeur J, 2008. The influence of habitat structure on sexual conflict over care in penduline tits *Remiz pendulinus*. *Ardea* 96:3-11.

van Dijk RE, Székely T, 2008. Post-fertilization reproductive strategies. In: Encyclopedia of Life Sciences (ELS). Chichester: John Wiley & Sons.

van Dijk RE, Szentirmai I, Komdeur J, Székely T, 2007. Sexual conflict over parental care in penduline tits: the process of clutch desertion. *Ibis* 149:530-534.

Van Roo BL, Ketterson ED, Sharp PJ, 2003. Testosterone and prolactin in two songbirds that differ in paternal care: The blue-headed vireo and the red-eyed vireo. *Hormones and Behavior* 44:435-441.

Weary DM, Lemon RE, Perreault S, 1992. Song repertoires do not hinder neighbor-stranger discrimination. *Behavioral Ecology and Sociobiology* 31:441-447.

Webb JN, Houston AI, McNamara JM, Székely T, 1999. Multiple patterns of parental care. *Animal Behaviour* 58:983-993.

Webb JN, Székely T, Houston AI, McNamara JM, 2002. A theoretical analysis of the energetic costs and consequences of parental care decisions. *Philosophical Transactions of the Royal Society of London B* 357:331-340.

Webster MS, Tarvin KA, Tuttle EM, Pruett-Jones S, 2007. Promiscuity drives sexual selection in a socially monogamous bird. *Evolution* 61:2205-2211.

Wedell N, Kvarnemo C, Lessells CKM, Tregenza T, 2006. Sexual conflict and life histories. *Animal Behaviour* 71:999-1011.

West SA, Gardner A, Shuker DM, Reynolds T, Burton-Chellow M, Sykes EM, Guinnee MA, Griffin AS, 2006. Cooperation and the scale of competition in humans. *Current Biology* 16:1103-1106.

West SA, Griffin AS, Gardner A, 2007. Evolutionary explanations for cooperation. *Current Biology* 17:R661-R672.

West-Eberhard MJ, 1984. Sexual selection, competitive communication and species-specific signals in insects. In: Insect communication (Lewis T, ed). New York: Academic Press.

Westneat DF, Stewart IRK, 2003. Extra-pair paternity in birds: Causes, correlates, and conflict. *Annual Review of Ecology and Systematics* 34:365-396.

Widemo F, Saether SA, 1999. Beauty is in the eye of the beholder: Causes and consequences of variation in mating preferences. *Trends in Ecology & Evolution* 14:26-31.

Wiggins DA, Part T, Gustafsson L, 1994. Correlates of clutch desertion by female collared flycatchers *Ficedula albicollis*. *Journal of Avian Biology* 25:93-97.

Wiklund C, Forsberg J, 1991. Sexual size dimorphism in relation to female polygamy and protandry in butterflies - A comparative study of swedish *Pieridae* and *Satyridae*. *Oikos* 60:373-381.

Wiley RH, Poston J, 1996. Perspective: Indirect mate choice, competition for mates, and coevolution of the sexes. *Evolution* 50:1371-1381.

Wingfield JC, Hegner RE, Dufty AM, Jr., Ball GF, 1990. The 'challenge hypothesis': Theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *American Naturalist* 136:829-846.

Wisenden BD, 1994. Factors affecting mate desertion by males in free-ranging convict cichlids (*Cichlasoma-Nigrofasciatum*). *Behavioral Ecology* 5:439-447.

Wong BBM, Candolin U, 2005. How is female mate choice affected by male competition? *Biological Reviews of the Cambridge Philosophical Society* 80:559-571.

Yasukawa K, 1981. Song repertoire in the redwinged blackbird *Agelaius phoenicerus*: A test of the Beau Geste Hypothesis. *Animal Behaviour* 29:114-125.

Zuk M, Ligon JD, Thornhill R, 1992. Effects of experimental manipulation of male secondary sex characters on female mate preference in red jungle fowl. *Animal Behaviour* 44:999-1006.

ACKNOWLEDGEMENTS

Undertaking the projects involved in this thesis was an interesting and great journey, and I could have not made a success of it if it was not for many people helping me to achieve this. I would like to express my special thanks to my two supervisors, ***Tamás Székely*** and ***Vilmos Altbäcker*** for their constant support throughout my PhD. I am extremely grateful to ***Tamás*** for introducing me into scientific thinking and approach to problems. I have learnt a great deal about all stages of research, from asking relevant scientific questions and designing studies that answer them to publishing the outcome in scientific journals of a high standard. He was always very supportive and accessible – no matter how deeply he was burried under his dozens of parallel projects running around the Globe. Constructive feedback came fast from him and his incredible productivity inspired me to desperately try to catch up with him. I can only hope that at least in some cases I managed to do so. His guidance not only helped me to achieve my goals in the above projects, but gave me a better understanding of how life may work in nature as well as in human societies. I have no doubts that Tamás's everlasting and infectious enthusiasm to understand animal behaviour and the delightful discussions and arguments over beers both contributed to this.

Vilmos has also been supporting me from the beginning of my doctoral work, first as the head of the Department of Ethology at Eötvös University, later as the head of Göd Biological Station where I was provided by facilities for my laboratory studies.

I am grateful to my excellent fellow researcher, ***René van Dijk*** for long discussions about sexual conflict, breeding system evolution and penduline tits. These many times contributed largely to clarifying my point of view. Working together in the field and sharing many projects with him throughout the last years convinced me without question that cooperation can be a very fruitful strategy.

I appreciate the help and support of the staff and students of the Department of Ethology at Eötvös University. Especially, I would like to thank to ***Antal Dóka*** for his kind assistance in various matters. I would also like to thank to all members of the Biodiversity Lab at the Department of Biology and Biochemistry, University of Bath for their creative scientific discussions and comments on my projects. I am also grateful to ***Helen Parkin*** at Bath University for assisting me regarding administrative problems.

I thank ***Zoltán Felker*** and ***Károly Csizmadia*** for their conscientious work of maintaing the captive population of Eurasian penduline tits in Göd Biological Station. I am also grateful to ***István Szentirmai, Lídia Mészáros, Gábor Bakacsi***, ***Béla Tokody, Orsolya***

Kiss and ***Miklós Beke*** and to all the students and fellow researchers participating in the fieldwork in Fehértó throughout the past years. Kiskunság National Park, Duna-Ipoly National Park, Hortobágy National Park and Szegedfish Kft. provided the necessary permissions to do my projects in Hungary, whereas ESKOM provided permission in South Africa. I thank ***András Kosztolányi*** for his help in resolving statistical problems many times. ***Xutong Yang*** did the molecular sexing of the captive Eurasian penduline tit population in ***Richard ffrench-Constant***'s laboratory at the University of Bath. ***Dóra Lengyel***, ***Jeroen van Dijk***, ***Alison Meredith*** and ***Sander Bot*** assisted data processing. Weather data was provided by ***Andrea Fülöp*** and the Hungarian Meteorological Service. I thank ***Laurence Hurst*** for helping to design the Monte Carlo simulation, and ***Freya Harrison*** and ***Barbara Taborsky*** for their comments. ***Johan van der Dennen*** wrote the MATLAB script to analyze the time-lapse video recordings. ***Csaba Daróczi*** provided me with his beautiful pictures about Eurasian penduline tits. I am grateful to the staff of Fakusz Bt. in Göd, who helped me to figure out solutions for many technical problems of designing and building the aviaries and experimental instruments.

I would also like to thank to ***Borbála Gerő*** and my family for their continous support and patience during sometimes difficult periods. Without their support, I can hardly imagine that I would have been succeeded.

My work was supported by a PhD studentship from Eötvös University, and a Marie Curie Fellowship from the European Commission (HPMT-CT-2001-00288). Research projects were supported by grants to Tamás Székely (Hungarian Scientific Research Fund: OTKA T031706 and T043390; Hungarian – South African Intergovernmental TÉT: OMFB-00564/2006; Biotechnology and Biological Sciences Research Council: BBSRC BBS/B/05788). The research leading to these results has received funding from the European Community's Sixth Framework Programme (FP6/2002–2006) under contract no. 28696.

PUBLICATIONS

Published papers and manuscripts included in the thesis

Published papers in international scientific journals

Pogány Á, Székely T, 2007. Female choice in the penduline tit *Remiz pendulinus*: The effects of nest size and male mask size. *Behaviour* 144:411-427. (*Chapter III*)

Pogány Á, Altbäcker V, Székely T, 2008. Male signalling and dominance in the penduline tit *Remiz pendulinus*. *Acta Zoologica Academiae Scientiarum Hungaricae* 54:191-199. (*Chapter IV*)

Pogány Á, Szentirmai I, Komdeur J, Székely T, 2008. Sexual conflict and consistency of offspring desertion in Eurasian penduline tit *Remiz pendulinus*. *BMC Evolutionary Biology* 8:242. (*Chapter VII*)

Manuscripts in revision and to be submitted

Pogány Á, Menyhárt O, DeVoogd TJ, Székely T. Acoustic signalling in Eurasian penduline tits *Remiz pendulinus*: The effects of intruder song on behaviour of resident males and females. *Manuscript* (*Chapter V*)

Pogány Á, van Dijk RE, Horváth P, Székely T. Sex differences in care provisioning do not explain female-biased uniparental care in the penduline tit *Remiz pendulinus*. *Manuscript submitted to Animal Behaviour* (*Chapter VI*)

van Dijk R, **Pogány Á**, Lloyd P, Komdeur J, Székely T. Sexual conflict predicts morphology and behaviour in two species of penduline tits. *Manuscript submittted to Behavioral Ecology* (*Chapter VIII*)

Conference presentations related to the thesis

Pogány Á, van Dijk R, Székely T, 2009. Sexual conflict in penduline tits: Hard working mothers and lazy fathers? Cooperation in Animal Societies, 10th Behavioural Ecology Meeting, Cluj Napoca, Romania.

Pogány Á, van Dijk R, Székely T, 2008. Sexual conflict in polygamous penduline tits: Sex differences in parental care do not explain female-biased care. 12th International Behavioral Ecology Congress, Ithaca, USA.

Pogány Á, van Dijk R, Komdeur J, Lloyd P, Székely T, 2008. Sexual conflict and cooperation in penduline tits: Influences on morphology and behaviour. Conflict and Cooperation in Animal Societies, Debrecen, Hungary.

van Dijk R, **Pogány Á**, Komdeur J, Lloyd P, Székely T, 2007. Sexual conflict *versus* cooperation between parents: A comparative study in penduline tits. 11th Congress of the European Society for Evolutionary Biology, Uppsala, Sweden.

Pogány Á, Szentirmai I, Komdeur J, Székely T, 2007. Sexual conflict between parents: Consistency in parental decisions of penduline tits. 11th Congress of the European Society for Evolutionary Biology, Uppsala, Sweden.

Pogány Á, van Dijk R, Horváth P, Székely T, 2007. The effects of sex-specific parental care in the penduline tit mating system. 10th Meeting of the Hungarian Ethological Society, Göd, Hungary.

Pogány Á, Székely T, 2007. Sexual conflict and cooperation in penduline tits. Meeting of the Hungarian Biological Society, Budapest.

Pogány Á, Szentirmai I, Komdeur J, Hurst L, Székely T, 2006. Consistency of offspring desertion in penduline tits *Remiz pendulinus*. 11th International Behavioral Ecology Congress, Tours, France.

Pogány Á, 2006. Experimental analysis of female choice in the penduline tit *Remiz pendulinus*. Edward Grey International Student Conference, Groningen, The Netherlands.

Székely T, **Pogány Á**, Szentirmai I, 2004. Sexual conflict over parental care: Environmental influence or genetic determination? Genes and Behaviour Gordon Conference, Ventura, California, USA.

Publications and conference presentations not related to the thesis

van Dijk R, **Pogány Á**, 2008. Strategic differences: The battle of the sexes in penduline tits. *Africa – Birds & Birding* 13:52-55.

Horváth Gy, Schäffer D, Molnár D, **Pogány Á,** 2006. Population study of small mammals in two river flat-type woods. *Natura Somogyiensis* 9:325-332.

Pogány Á, Szentirmai I, Székely T, 2005. Variance of sexually selected traits in relation to reproductive success in European penduline tits *Remiz pendulinus*. XXIX. International Ethological Conference, Budapest, Hungary.

Horváth Gy, **Pogány Á**, Hamburger K, Sárkány H, 2004. New population data from trapping studies of the protected field vole *Microtus agrestis* (Linnaeus, 1761) in the Kis-Balaton marshland area**.** *Állattani Közlemények* 89:27-35.

Horváth Gy, **Pogány Á**, Hamburger K, Schäffer D, 2004. Distribution of the protected field vole, *Microtus agrestis* (Linnaeus, 1761) in Hungary, based on data collected up to 1999. *Természetvédelmi Közlemények* 11:597-606.

Horváth Gy, **Pogány Á**, Hamburger K, Schäffer D, 2002. The range of the protected field vole, *Microtus agrestis* (Linnaeus, 1761) in Hungary and its synbiological study in the Kis-Balaton area. 1st Hungarian Biological Conference in Environmental Protection, Sopron, Hungary.

Horváth Gy, Schäffer D, Hamburger K, Molnár D, **Pogány Á**, 2001. Population study of small mammals in two river flat-type woods. II. International Dráva Conference, Pécs, Hungary.

Konecsni T, **Pogány Á**, Bui A, Dergez T, Szokisboris P, Szécsényi M, Funa K, Muhr C, Kilár F, 1999. Spatial distribution of hormone mRNAs in a pituitary gland. 8th Semmelweis Symposium: Past, Present and Future in Neuroendocrinology, Budapest, Hungary.

SUMMARY

Sexual reproduction is a complex interaction between males and females when they cooperate to produce offspring. However, each sex may increase its reproductive success independently, regardless of the benefit or cost to its mate. Sexual selection therefore involves two processes; traits may evolve by classical sexual selection, when evolutionary change in one sex is also beneficial to the other, but sexual conflict may lead to the evolution of traits with antagonistic effects on the reproductive success of the sexes.

In this PhD thesis I investigated two processes of sexual selection in penduline tits (*Remizinae*) using field and laboratory experiments. Penduline tits are small passerine birds that live in a range of habitats from resource-rich marshlands to resource-poor semi-deserts. Their breeding system is variable, and I investigated two species with contrasting breeding strategies: Eurasian penduline tits *Remiz pendulinus* have intense sexual conflict with uniparental care and biparental desertion, whereas Cape penduline tits *Anthoscopus minutus* are cooperative with biparental care and facultative cooperative breeding.

I showed that mask size in Eurasian penduline tits is sexually selected *via* female choice, and it does not appear to signal the contesting abilities of males. Furthermore, a cost is associated with this trait in females, because males with large masks deserted the female and her clutch more frequently than males with small masks. This suggests that sexual conflict is a plausible explanation for the evolution of mask size. However, nest size is not sexually selected, whereas song repertoire is an honest signal in male contests.

The resolution of sexual conflict over care is biased towards males in Eurasian penduline tits: they care less frequently for the clutches than females. By comparing male and female parental behaviour and success of their broods, I showed that the sexes provide comparable care, so that the biased care pattern is not driven by a sex difference in parental abilities. There are, however, sex differences in the flexibility of parental decisions, since desertion behaviour of females is consistent between their subsequent nests, whereas male desertion is influenced by environmental changes.

By comparing the morphology and behaviour of Eurasian and Cape penduline tits, I found results consistent with predictions of sexual conflict theory. Eurasian penduline tits exhibited more intense sexual dimorphism and they were less cooperative in nest-building than Cape penduline tits. Taken together, my thesis strongly suggests that sexual conflict is a powerful evolutionary force, and together with classical sexual selection it has a fundamental influence on male-female coevolution and breeding systems in penduline tits.

ÖSSZEFOGLALÓ

Az ivaros szaporodás olyan összetett kölcsönhatás, amelyben a hím és nőstény együttműködik az utódok létrehozásáért. Mindkét fél növelheti azonban szaporodási sikerét attól függetlenül, hogy ez előnnyel vagy hátránnyal jár párja számára. A szexuális szelekció így két folyamatot foglal magába: a klasszikus szexuális szelekciót, amikor az egyik nemnél bekövetkező változás a másik nem számára is előnyös, és a szexuális konfliktust, amikor a jelleg evolúciója ellentétes hatású a két nem szaporodási sikerére.

Az értekezésemben laboratóriumi és terepi kísérletekkel vizsgáltam a szexuális szelekció két folyamatát függőcinegéken (*Remizinae*). A függőcinegék kistestű énekes madarak, élőhelyeik a forrásokban gazdag lápoktól a szegényes félsivatagokig terjednek. A szaporodási rendszerük szintén változatos. Két ellenkező szaporodási stratégiájú fajt vizsgáltam: az Eurázsiai függőcinegét *Remiz pendulinus* szexuális konfliktus, egyszülős gondozás és biparentális dezertálás, míg a Dél-Afrikai függőcinegét *Anthoscopus minutus* kooperáció, kétszülős gondozás és fakultatív kooperatív költés jellemzi.

Kimutattam, hogy a szemsáv mérete az Eurázsiai függőcinegénél nőstény válasz által szelektált, de nem jelzi a hímek kompetitív képességét. A nagyobb szemsávú hímek gyakrabban dezertálták párjukat és a fészekaljat, így a jelleg szelekciója költséges a tojók számára, és ez arra utal, hogy szexuális konfliktus által fejlődik. A fészekméret nem szexuálisan szelektált, a repertoár méret viszont őszinte jelleg a hím-hím versengésben.

A szülői gondozás fölötti konfliktus kimenetele az Eurázsiai függőcinegénél a hímek előnyére tolódott el, mivel a hímek ritkábban gondozzák a fészkeket. Hím és tojó függőcinegék gondozását és fészekaljaik sikerét összehasonlítva kimutattam, hogy a nemek gondozásában nincs eltérés, így az eltolódott gondozási mintázatot nem a szülői képességek ivari különbsége okozza. Eltérő azonban a nemek rugalmassága a szülői gondozást illetően, mert a tojók következetesek dezertáló viselkedésükben az egymást követő fészkeiknél, amíg a hímek dezertálását befolyásolja a környezetük.

Az Eurázsiai és Dél-Afrikai függőcinegék morfológiáját és viselkedését összehasonlítva a szexuális konfliktus elmélet predikcióival egybevágó eredményeket kaptam. Az Eurázsiai függőcinegére erős ivari dimorfizmus jellemző és kevésbé kooperatív fészeképítés, mint a Dél-Afrikai társára. Mindent egybevéve az értekezésem eredményei arra utalnak, hogy a szexuális konfliktus egy erőteljes evolúciós folyamat, ami a klasszikus szexuáls szelekcióval együtt hozzájárul a függőcinegék hímjeinek és nőstényeinek koevolúciójához és szaporodási rendszerük fejlődéséhez.

APPENDIX

1. Manual for keeping Eurasian penduline tits in captivity

Room demands and decorations

Eurasian penduline tits involved in the above studies were caught in the wild, thus I tried to house them as spaciously as possible. During autumn and winter (i.e. outside the breeding period), Eurasian penduline tits flock in the wild thus males and females were kept together in five indoor aviaries (Figure A.1). During their natural breeding season (between early April – August), males behave aggressively towards each other so our captive population was housed as male-female pairs also in six outdoor aviaries besides the indoor aviaries (Figure A.2 and A.3). The sizes of rooms were 5.1 m x 3.4 m x 2.4 m and 3.4 m x 3.4 m x 2.4 m (indoor) and 3 m x 2 m x 2.4 m (outdoor) each, resulting in total aviaries of 166.6 m^3 (density: one Eurasian penduline tit per 4.6 m^3) and 253 m^3 (density: one individual per 7 m^3) during winter and summer, respectively. Individuals were swapped between indoor and outdoor aviaries in summer, so that spent approximately half of their time each indoor and outdoor.

Figure A.1. Eurasian penduline tits flock during winter, and one can keep many of them together outside the breeding season.

Eurasian penduline tits live in wetland habitats, mainly reed marshes and gallery forests. To mimic their natural habitats as closely as possible, I used reed as the main decoration. Commercially available reed mats were mounted on the walls and reed was collected from the river banks of the nearby Danube that was placed in the corners of the rooms (Figure A.2). Also, reed was erected in plastic flower pots, so that they could be placed in the middle of the room. The pot was first filled up with light concrete (a mixture of polystyrene balls, some cement and water) and reeds were placed in them. The floor of indoor aviaries was covered by wood shavings.

Figure A.2. One of the five purpose-built indoor aviaries decorated according to the needs of penduline tits.

Besides the reeds, Eurasian penduline tits spend lots of time on hanging branches of trees, therefore I provided ample resting sites in indoor aviaries by stretching a network of cords from approximately 20 cm of the ceiling from which poplar, willow and ivy branches were hung. Outdoor aviaries were also planted with real shrubs and bamboo reed and in each, a small pond (2 m x 1 m x 0.4 m) was dug (Figure A.3). The bottom of the pond was covered by felt (to protect foil against roots) and by a layer of thick plastic foil. These lakes were filled up with water from the nearby small lake using a hydro-pump.

Indoor aviaries were cleaned every other month by replacing the decoration (reed, wood branches and wood shavings) with fresh decoration. Water in the ponds of outdoor aviaries was also changed using the pump during summer when longer periods without rain prevented the water to refresh naturally.

Figure A.3. Outdoor penduline tit aviaries in Göd Biological Station, Eötvös University. The complexum consists of six separable compartments, each 2 m x 3 m x 2.4 m in dimensions.

Technical details and protection

Both indoor and outdoor aviaries were sluiced by double doors in order to prevent birds from escaping or damage when we entered the aviaries (Figure A.4). Also, windows were protected by double layers of wire mesh and metal mosquito net, allowing the airing of the aviaries. From spring until the end of autumn, these windows were kept open. The inner side of the windows were also covered by wire mesh some 3 cm from the glass to prevent birds from bumping to the glass and instead providing landing sites (Figure A.5).


Figure A.4. Double doors and two layers of wire mesh and mosquito net on the windows prevented penduline tits from escaping or damage.

All indoor aviaries were equipped with time-controlled light tubes and mixed light sources (Tungsram 160 W HMLI, GE Hungary Rt., Hungary), which allowed us to increase and modify light intensity and photoperiod in indoor aviaries. A 60 W blue light tube was used all night long to provide twilight conditions. Indoor aviaries were also equipped with electric oil radiators so that a constant 14-15 Cº was kept during winter months (Figure A.5).

Since decoration of the aviaries included highly flammable elements (e.g. reed, wood branches), I paid extra caution to fire prevention. First, electricity and fuses of all aviaries were checked and sockets were replaced by insulated ones, mounted on a special insulation sheet that prevented any fire caused by electric sparks (Figure A.5). Second, all electronic devices (e.g. lamps, radiators) that were placed within the aviaries were covered and protected from possible short circuits caused by the excrement of the birds (Figure A.5). These covers also protected the birds from injuries caused by hot lamp bulbs or radiators.

Figure A.5. Electricity and electrical devices were protected by insulation from flammable environment and the excrements of birds that could cause short circuits.

Catching penduline tits in the aviaries

To catch penduline tits in the aviaries we used a 1.5 m x 1 m mist net attached to a frame. The mist net was hung in the middle of the aviary, and the targeted female was herded into it. This method allowed us to catch the females efficiently without injuring them.

Diet

Penduline tits received protein-rich diet with vitamin supply (Lisovit R-combi, Pentarex Bt, Hungary). The special diet consisted of meal-worms, insectivorous dry bird food mixture (Prosecto, J. E. Haith, UK), minced beef with carrots and boiled eggs, and cat food (Hill's Feline Prescription Diet A/D, Hill's Pet Nutrition Inc., Topeka, Kansas, USA). Food and fresh water were changed daily and were available *ad libitum* all days including experimental days in the apparatus.

2. Supplementary information to Chapter VI

Table A.1. The number of Eurasian penduline tit nests used in statistical analyses. Sample sizes are given for each year separately, total number of nests (or broods), and male-cared (MC) and female-cared (FC) clutches. See Methods of *Chapter VI* for details.

Year		Incubation behaviour	Feeding frequency	Offspring survival	Nestling size
2002	MC			9	8
	FC			31	26
	Total			40	34
2003	MC			6	6
	FC			21	19
	Total			27	25
2004	MC			5	3
	FC			36	28
	Total			41	31
2005	MC	1	3	1	
	FC	9	8	11	
	Total	10	11	12	
2006	MC	4	4	7	
	FC	9	9	15	
	Total	13	13	22	
2007	MC	4	3	5	
	FC	2	3	3	
	Total	6	6	8	
Total	**MC**	**9**	**10**	**33**	**17**
	FC	**20**	**20**	**117**	**73**
	Total	**29**	**30**	**150**	**90**